

Stephen, Norman H. (1983) *Studies on the behaviour of bracken control chemicals in plant-soil systems*. PhD thesis.

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STUDIES ON THE BEHAVIOUR OF
BRACKEN CONTROL CHEMICALS IN
PLANT-SOIL SYSTEMS

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Thesis presented for
the degree of
Doctor of Philosophy
March, 1983

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ACKNOWLEDGEMENTS

I am very grateful to Dr. H.J. Duncan for his supervision of the work described in this thesis. His advice and encouragement throughout the project were of great value to me.

I am also indebted to Dr. G.T. Cook for his co-operation and discussion, as well as his assistance with the field trials.

I wish to thank staff and colleagues in the Agricultural Chemistry Section, both past and present, who have been of valuable assistance in many ways. In addition, I thank Mr. T.C. Aitchison for advice on statistical matters and Messrs. M. Beglan and R. Munro for their photographic work.

The provision of bracken trial sites and the co-operation given by Mr. J. Pirie, Laighpark Farm, Milngavie, is appreciated.

The financial support of the Department of Agriculture and Fisheries for Scotland in the form of a post-graduate studentship is gratefully acknowledged.

I wish to thank Mrs. June Anthony, who typed this thesis, for her kindness and efficiency.

Finally, I would like to thank my wife, Lisbeth, for her patience and encouragement throughout the work and preparation of this thesis.

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DECLARATION

Preliminary reports of much of the data forming chapters 2, 4, 5 and 6 have already been published, see - Stephen et al. (1979; 1980; 1980a) and Cook et al. (1981; 1982).

SUMMARY

This thesis is principally an investigation on behavioural aspects of bracken control herbicides in some plant and soil systems with a view to improving their effectiveness. The main chemicals investigated were asulam and aminotriazole. As the work developed broader aspects were studied, including the behaviour of iodide and thiocyanate in plants. The work can be sub-divided as follows:-

1. A brief discussion of bracken and its control was made with some emphasis being placed on its control by systemic herbicides and possible approaches to improving their efficiency.
2. An investigation into the mechanism of action of asulam in plants. The findings can be summarized as follows:-

Preliminary work involving IAA-oxidase assays and plant growth studies revealed an interesting interaction between *p*-aminobenzoic acid and asulam which indicated sulphonamide-type activity for asulam. A series of experiments were carried out whereby a range of species sown in beakers containing vermiculite were treated with asulam solutions. This resulted principally in stunting of the root system which could be partially or totally overcome by the simultaneous addition of either *p*-aminobenzoic acid or folic acid. Compounds related to *p*-aminobenzoic acid had no such activity, indicating the specificity of the antagonism. The results led to the conclusion that a possible mechanism of action of asulam is the inhibition of folic acid synthesis resulting in impairment of biological methylations and hence inhibition of protein and nucleic acid synthesis. The selectivity of asulam may be due to differential

p-aminobenzoate or folate concentrations.

3. An investigation into the possibility of achieving pre-emergence activity for asulam in bracken control. The results can be summarized as follows:-

An appraisal of asulam soil behaviour was made, whereupon it was considered that the use of additives would probably be required to regulate movement and prolong persistence for sufficient quantities to achieve contact with the rhizome buds.

Asulam adsorption experiments, carried out using surfactant solutions and three acid-organic soils found under bracken, revealed that, on the whole, the anionic surfactants employed (sodium dodecyl sulphate and sodium dodecylbenzene sulphonate) significantly reduced asulam adsorption, whilst cetyl trimethylammonium bromide (cationic surfactant) significantly increased adsorption. These trends were reflected in leaching experiments using a thick layer soil plate method. Most surfactant treatments decreased persistence of asulam in non-leaching degradation experiments, sodium dodecylbenzene sulphonate being an exception. No carbamate-derived additive had any effects on asulam persistence.

In a field trial, pre-emergence application of asulam (6.7 kg/ha) had no effect on bracken. The use of 1% cetyl trimethylammonium bromide or 1% sodium dodecylbenzene sulphonate in the spray formulations had no beneficial effect.

4. An investigation into the use of ammonium thiocyanate and other additives in bracken control formulations to reduce herbicide detoxification. The results can be summarized as follows:-

Established work had shown that scorching of bracken fronds by aminotriazole was a result of detoxification via a free radical mechanism and led to subsequent poor performance due to decreased translocation. A bioassay, involving floating bracken leaflets on aminotriazole solutions, was developed for assessing the effects of the free radical scavenger ammonium thiocyanate on aminotriazole scorching and assessing the activities of other potential additives. The results indicated that the concentration of ammonium thiocyanate, rather than the 1:1 ratio with aminotriazole at present used commercially, is the critical factor in determining the reduction in scorching. Further results revealed that the concentration of ammonium thiocyanate required could vary depending on the environment under which the bracken was growing. In a field experiment using 8.4 kg/ha aminotriazole and ammonium thiocyanate at a range of ratios from 1:0 to 1:1.25, only the 1:0.5 aminotriazole to ammonium thiocyanate gave significantly greater control than the aminotriazole alone after 3 years (74% control). Of other potential additives tested in the bioassay, thioacetamide was selected as promising for aminotriazole formulations, however, in a field trial it proved ineffective at the level used.

Successful incorporation of asulam into the scorching bioassay proved difficult. In a field trial, an ammonium thiocyanate-asulam mixture (10:1) had no beneficial effect over asulam alone (2.2 kg/ha) at 2 spraying dates.

Preliminary experiments involving the use of dock and potato leaflets, as material for the scorch bioassay, produced promising results for the tailoring of aminotriazole-ammonium thiocyanate formulations for improved effective control.

5. An investigation into the selective herbicidal activity of iodide.

The results can be summarized as follows:-

In initial studies, the rate of iodide oxidation in an in-vitro hydrogen peroxide/horseradish peroxidase system was found to be reduced by the addition of ammonium thiocyanate. The selective herbicidal activity of sodium iodide towards dwarf bean, pea, kale and cabbage was tested while the potential thiocyanate content of these plants was measured. A good relationship between increasing thiocyanate content and increasing resistance to iodide was observed. Further studies achieved limited success in conferring resistance to iodide in bean with thiocyanate additions, while the mechanism of inhibition of thiocyanate in the enzyme system was found to be mainly through chemical reduction of the iodine product - the toxic moiety. Evidence was gathered to suggest that other compounds present in plants, including glucosinolates, thiols and ascorbate, and some pesticidal additions, may contribute to the overall selectivity through their effect on iodine formation and/or persistence.

6. An investigation into the mode of action of iodide and thiocyanate in plants. The results can be summarized as follows:-

Whole plant studies using dwarf bean reaffirmed that of the halides and pseudohalide thiocyanate, only iodide and thiocyanate possess defoliant properties. Established work had indicated their possible involvement with IAA (indole-3-acetic acid). In-vitro enzyme studies and photochemical and chemical oxidations of IAA, suggested that IAA destruction is not through a direct interaction between IAA and the halide and pseudohalide but depends on their conversion to the halogen

or pseudohalogen which can then destroy IAA. This may be accomplished by the peroxidase enzyme system for iodide only and does not provide a mechanism for thiocyanate activity. However, both iodide and thiocyanate have chemical oxidation potentials suitable for their conversion to the halogen or pseudohalogen respectively via the photosynthetic apparatus. Hence, this provided an explanation for their activity and the apparent inactivity of bromide, chloride and fluoride which would be oxidised very slowly or not at all.

7. Some conclusions as to the outcome of the studies were drawn and some suggestions made for further work.

A list of abbreviations for names (other than chemical) used in this thesis

abs.	absorbance
A.D.E.	air dry equivalent
CEC	cation exchange capacity
cm	centimetre
c.m.c.	critical micelle concentration
c.v.	cultivar
DF	degrees of freedom
E _o (v)	oxidation potential (in volts)
g	gram
h	hour
ha	hectare
kg	kilogram
l	litre
lb	pound
LSD	least significant difference
m	metre
M	molar unit
m ²	square metre
mg	milligram
min	minute
ml	millilitre
mm	millimetre
N	normal unit (concentration)
nm	nanometre
no.	number
P	statistical probability
pp.	pages
ppm	parts per million
p.s.i.	pounds per square inch
ref.	reference
r.h.	relative humidity

rpm	revolutions per minute
sec	second
sp	species (singular)
spp.	species (plural)
t	tonne
t.l.c.	thin-layer chromatography
v	volume
w	weight
%	percent(age)
°C	degrees Celsius
°	degrees (angle)
<	less than
>	more than
µg	microgram
µl	microlitre

note. Abbreviations for the name of units are the same for singular and plural.

A list of common names and abbreviations for herbicides and other chemicals used in this thesis.

aminotriazole	3-amino-1,2,4-triazole
asulam	methyl[4-aminobenzenesulphonyl]carbamate
atrazine	2-chloro-4-ethylamino-6-isopropyl-amino-1,3,5-triazine
barban	4-chlorobut 2-ynyl 3-chlorophenylcarbamate
benomyl	methyl 1-(butylcarbonyl)benzimidazol-2-ylcarbamate
bromoxynil	3,5-dibromo-4-hydroxybenzonitrile
carbaryl	1-naphthyl methylcarbamate
CDDA	N,N-diallyl-2-chloroacetamide
CEPA	2-chloroethylphosphonic acid
cetab	cetyl trimethylammonium bromide
CIPC	isopropyl 3-chlorophenylcarbamate
4-CPA	(4-chlorophenoxy)acetic acid
2,4-D	(2,4-dichlorophenoxy)acetic acid
dalapon	2,2-dichloropropionic acid
diazinon	O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate
dicamba	3,6-dichloro-2-methoxybenzoic acid
diquat	1,1'-ethylene-2,2'-bipyridyldiylum ion
diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea
EPTC	5-ethyl dipropylthiocarbamate
fluometuron	1,1-dimethyl-3-(trifluoromethylphenyl)urea
folic acid	N-[4-[[2-amino-1,4-dihydro-4-oxo-6-pteridiny]methyl]amino]benzoyl]-L-v glutamic acid

glyphosate	N-(phosphonomethyl)glycine
HRPO	horseradish peroxidase E.C.1.11.1.7
IAA	indole-3-acetic acid
ioxynil	4-hydroxy-3,5-di-iodobenzonitrile
isoproturon	3-(4-isopropylphenyl)-1,1-dimethylurea
lindane	1 α ,2 α ,3 β ,4 α ,5 α ,6 β -hexachlorocyclohexane
MCPA	(4-chloro-2-methylphenoxy)acetic acid
nabam	disodium ethylenebis(dithiocarbamate)
PABA	4-aminobenzoic acid
paraquat	1,1'-dimethyl-4-4'-bipyridyldiylum ion
parathion	O,O-diethyl O-4-nitrophenyl phosphorothioate
picloram	4-amino-3,5,6-trichloropicolinic acid
propanil	N-(3,4-dichlorophenyl)propionamide
propham	isopropyl phenylcarbamate
SDBS	sodium dodecylbenzene sulphonate
SDS	sodium dodecyl sulphate
trifluralin	2,6-dinitro-N,N-dipropyl-4-trifluoromethyl- aniline
zineb	zinc ethylene-1,2-bisdithiocarbamate

CHAPTER 1

INTRODUCTION

1.1 Background

The background and basic aim of the studies of this thesis was to improve the efficiency of herbicides for the control of the weed bracken (Pteridium aquilinum L. Kuhn). The investigations developed into a study of aspects of herbicide behaviour with relevance to a wide range of plant and soil systems. The topics studied are quite diverse and it could be considered that most, if not all, of them stand well in their own right. However, it was felt that a brief introduction to the bracken problem would be of value at this stage to indicate the approach behind the various topics investigated.

There would be little of a novel nature to be gained from a full literature review covering the bracken plant and the problems associated with its control since its biology, ecology and control, etc., have been well covered in publications over the years. Braid (1959) has given an exhaustive review of the literature on bracken and its control up to 1959. Recent updates on this, with particular relevance to the use of systemic herbicides for control, have been made by Babiker (1976), Fletcher and Kirkwood (1979) and Williams (1980). Articles covering many aspects of its biology, taxonomy, ecology, distribution, toxicity and control can also be found in the published proceedings of two recent symposia devoted to bracken, see Perring and Gardner (1976) and Fletcher and Kirkwood (1982). Thus, only a brief

discussion of bracken and its control is given below. Emphasis is placed on some aspects of its control by systemic herbicides, highlighting specific points relevant to the investigations carried out herein.

1.2 The Bracken Problem

Bracken is an unpalatable, poisonous fern with world-wide distribution in humid and sub-humid regions (Robocker, 1971). It is one of the most persistent weeds present in upland pastures in the UK, being particularly a problem on land inaccessible to the plough and occupying soils which are potentially good for pasture land (Tansley, 1949). It dominates at least 200,000 ha of hill land in Scotland (Taylor, 1980), making shepherding difficult and causing stock poisoning (Williams, 1980). It also presents a weed problem to young trees in forestry situations (Biggin, 1982).

Although the life-cycle of bracken does possess a means of sexual reproduction via spores (Conway, 1952), the main spread of bracken in established colonies is by vegetative means by way of its extensive underground rhizome system (Conway, 1959). The rhizomes can penetrate to a depth of 0.75 m (Braid, 1957) and weigh 50-100 t/ha (Fletcher and Kirkwood, 1962). The rhizome system presents a difficult target for the control of bracken by chemical or mechanical means. It contains considerable carbohydrate reserves (Williams and Foley, 1976) and a vast number of buds - approximately 1-2 million/ha - only a proportion (20-40%) of which develop into frond buds in one season (Hodgson, 1963), the remainder staying dormant for long periods, up to 18 years (Smith,

1928). Practices which fail to destroy the rhizome system, fail to provide effective control of bracken (Hodgson, 1964).

1.3 Bracken Control

Before the advent of modern herbicides, cultural methods were the only feasible means of control (Robocker, 1971). Autumn ploughing, on suitable ground, is usually an effective means of control, the exposure to winter temperatures contributing here (Conway, 1959). On hillsides and rough land ploughing was not always possible so cutting or burning the fronds over a number of years, so as to deplete the rhizomes of their starch reserves, was the method commonly employed (Fletcher, 1978). Despite government grant-aid schemes to assist cutting and follow-up treatment, the acreage of land treated by this notoriously slow and laborious method has declined progressively to negligible levels due to the unsatisfactory results of cutting programmes, shortage of labour and the advent of potentially promising systemic herbicides (Fletcher and Kirkwood, 1979).

Although susceptible to a few diseases (Braid, 1959), the biological control of bracken does not appear to be feasible at present (Fletcher and Kirkwood, 1979), unless one considers that the heavy stocking of cattle and sheep, integrated with a sowing and fertilising programme, falls into this category.

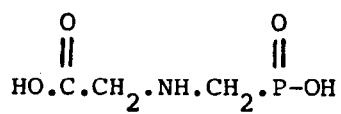
Many foliar applied contact herbicides have been tried and discarded for use in bracken control. Such chemicals have included sulphuric acid, sodium borate, sodium chlorate, ammonium sulphamate, diquat and paraquat (see Babiker, 1976; Fletcher and Kirkwood, 1979).

These chemicals act only on the growing fronds, thus can only be as effective as cutting and probably more expensive (Jarvis, 1974).

The development of systemic herbicides, which offered the possibility of attacking the rhizome chemically, led to an extensive effort in bracken control research (Babiker, 1976). The structure of some of the more successful chemicals which have been employed are shown in figure 1.1.

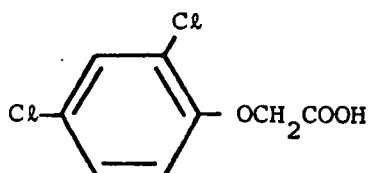
Application can be by foliar means or to the soil before frond emergence. Unless specifically stated otherwise, all further discussion in this chapter is concerned with foliar-applied systemic action. Common features associated with most, if not all, of the chemicals used in this way have been inconsistencies in performance at different sites, in different years, and the ability of the weed to regenerate after treatment (Jarvis, 1974; Babiker, 1976). After early promise, poor overall performance has led to the withdrawal of chemicals such as 2,4-D, MCPA, 4-CPA, dicamba, picloram and aminotriazole as foliar applied herbicides recommended for bracken control. The most consistent and widely used herbicide so far has been asulam (methyl[4-aminobenzene-sulphonyl]carbamate), indeed it is the only chemical which warrants a 50% government grant towards the cost of spraying coupled with fertilizer application. The herbicide glyphosate also produces reasonable results and is recommended (although not grant-aided) for non-selective non-crop situations (Fryer and Makepeace, 1978).

The importance of after-spray and general hill management, in determining the overall success and economic viability of a bracken clearance programme, cannot be overstated. Reviews on the economics of

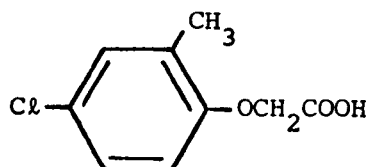


OH

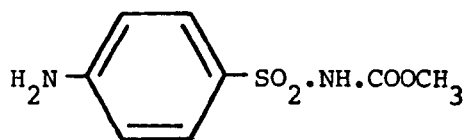
GLYPHOSATE



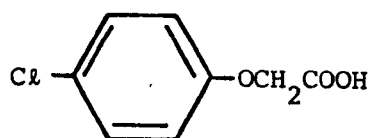
2,4-D



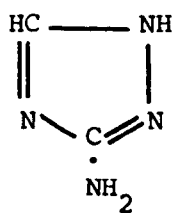
MCPA



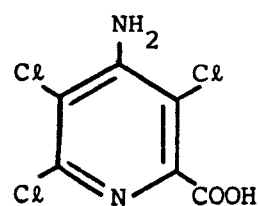
ASULAM



4-CPA



AMINOTRIAZOLE



PICLORAM

Figure 1.1 Structures of some herbicides used in bracken control.

bracken control have been made by McCreath (1976; 1982), while valuable discussion on after-spray management strategies have been made by Jarvis (1974) and Williams (1980). It is generally agreed that, in the absence of after-treatments, bracken recolonisation follows a logarithmic pattern, thus, relatively minor variations in the level of control in the year after spraying can therefore become much more important in subsequent years (Williams, 1980). The ideal chemical has still not been found since, after spraying at economic rates and without the reliance on considerable post-application management efforts, there is no evidence of 100% kill nor the failure of bracken to regenerate. Thus, one talks of controlling bracken rather than killing it with chemicals. The role of the herbicide scientist is to maximise the effect from chemical treatment, economically and under the widest range of situations, to minimise the need to rely on after-care.

The variable effects achieved with chemical control have been illustrated by Babiker (1976) who, in summing up the performance of 4-CPA, dalapon, aminotriazole and asulam, drew the following common points associated (more or less) with all four chemicals:

- 1) A period of maximum susceptibility was observed. The best results were generally obtained when the majority of fronds had just completed the unfurling process. Early or late application could lead to inadequate control.
- 2) Rainfall after application was found to reduce herbicidal activity.
- 3) Marked variations in effectiveness of the herbicide was observed and it was more pronounced at lower rates.
- 4) Regeneration of bracken took place. It varied with site, dosage rate and timing of application.

These common points suggest that at least most factors responsible for the variable effectiveness of these herbicides could be traced to common origins, although differences in movement and intrinsic phytotoxicity of the individual herbicides cannot be overlooked (Babiker, 1976). A major criteria determining the efficiency of long-term control by systemic herbicides is their ability to reach the rhizome and its associated buds (Hodgson, 1964). Such ability will be influenced by the efficiency of their uptake (including spray retention) and translocation. Once at the site of action, the inherent toxic activity of the particular herbicides involved become important.

A discussion of Babiker's point 1 (see above) may best illustrate some of the processes the foliar applied systemic chemical has to go through and where many of the problems associated with their use lie. The importance of time of application for efficient control by foliar applied systemic herbicides has been emphasised by many workers (Erskine, 1960; Hodgson, 1960; Soper, 1972; Volger and Rösger, 1972; Veerasekaran et al., 1977). Under UK conditions, the recommended time to spray bracken is when the fronds have fully unfurled and before they begin to senesce (Williams, 1980). This period of maximum susceptibility to post-emergence herbicide treatment is dependent on herbicide retention, penetration, translocation and rhizome bud activity being optimal:

Posture and stage of development of the frond is important; a large area for spray interception and retention will be provided by fully expanded fronds (Babiker, 1976):

Penetration of asulam (Veerasekaran et al., 1976; 1977) declined with increasing frond age, probably due to increasing thickness of the cuticle limiting entry:

It is widely accepted that translocation of herbicides takes place predominantly in the phloem and that they are closely linked with movement of sucrose from the region of assimilate synthesis (source) to the region of utilisation (sink) (Conway, 1959; Jarvis, 1974; Veerasekaran et al., 1976). After full frond expansion, translocation is predominantly basipetal, only diminishing as a result of decreasing day length and eventual senescence and death of the fronds (Whittle, 1964):

The growth of rhizome apices and buds act as active sinks, encouraging herbicide accumulation. Growth in these centres begins generally in late-June and late-July, respectively, and continue only until the early autumn (Watt, 1950; Conway and Stephens, 1954).

In Scotland, the period of optimum spraying is a 4 week period from approximately mid-July to mid-August. Weather conditions (rainfall, wind velocity, humidity, etc.) will also exert considerable influence on the quantity of chemical retained (Fletcher and Kirkwood, 1962; Babiker, 1976) and taken up by the fronds (Holroyd et al., 1970; Brown and McKenzie, 1972; Cook and Duncan, 1978). The short spraying season is often accompanied by uncertain weather conditions. When coupled to the fact that variations in the stage of maturation caused by site (aspect, soil depth, degree of exposure, etc.) and seasonal differences can also occur (Braid, 1947), this makes for a need to achieve the maximum performance from the applied chemicals which are

obliged to operate following stringent rules and under a wide range of variable situations.

1.4 Possible approaches to improved bracken control by herbicides

Improvement of spray retention may be achieved by optimising application techniques (nozzle type, spraying height, droplet size, etc.) (Babiker, 1976; Williams, 1980) and by incorporation of surface active agents (surfactants) - Babiker, 1976. The use of additives to improve the foliar uptake of the herbicides has received a great deal of attention. The addition of surfactants has been shown to increase the penetration of asulam (Babiker and Duncan, 1974; Veerasekaran et al., 1977) and aminotriazole (Babiker and Duncan, 1975; Cook, 1979) into bracken fronds. Various workers failed to find improved effectiveness in control to result from the use of surfactants in bracken control formulations (Holroyd and Thornton, 1978; Cook, 1979; Cook et al., 1982). However, the addition of diesel oil and emulsifier has been shown to improve the effectiveness of asulam in New Zealand (Preest, 1975). In this country, formulation of asulam with a non-ionic surfactant showed slightly improved control over a 4 year period (Veerasekaran et al., 1978). Recently, the addition of Dessipron (B.P.) an emulsifiable oil, has been recommended by the manufacturers to improve the uptake of asulam, especially as an insurance when early rain follows application (Heywood, 1982). The use of foliage-applied herbicides may not be completely satisfactory because of the metabolic inactivity of a large proportion of the buds in various rhizome branches. Inactive buds do not act as metabolic sinks and consequently fail to receive a toxic concentration

of the herbicide. Pre-treatment with low levels of the plant growth regulator CEPA, an ethylene generator, has been used in an attempt to stimulate activity of rhizome buds and thus enhance asulam accumulation. CEPA increased basipetal translocation of ^{14}C label in plants treated with ^{14}C -asulam, however, no significant difference in field control resulted (Veerasekaran et al., 1976). Gibberellic acid pre-treatment also induced bud activity but had no effect on the overall efficiency of aminotriazole (Hodgson, 1964) or asulam (Veerasekaran et al. 1976).

The efficiency of foliar applied translocatable herbicides depends not only on their uptake and translocation but also on their metabolism by various degradative processes in the plant (Veerasekaran et al., 1976) and their intrinsic activity once at the site of action (Babiker, 1976).

Biochemical and photochemical degradation of asulam and the binding of asulam to plant constituents has been demonstrated (Veerasekaran, 1975; Veerasekaran et al., 1976; 1977; Cook, 1979). Evidence has been gathered in this laboratory (see Babiker, 1976; Cook, 1979; Cook and Duncan, 1979) that the inconsistent results on bracken achieved by aminotriazole (Erskine, 1960; Hodgson, 1960; Kirkwood and Fletcher, 1961), and possibly other herbicides, may be as a result of different degrees of detoxification via a free radical oxidation and subsequent conjugation to other plant constituents. This would render the aminotriazole unavailable for translocation to the rhizome system. The detoxification reaction of aminotriazole is manifested as frond scorch after spraying, the formation of which is inversely related to the subsequent degree of control. The carefully selected and controlled

use of free radical scavengers as additives could hence enhance the concentration of aminotriazole available for translocation.

Not a great deal of detail is known concerning the mode of action of most of the chemicals which have shown some degree of success in controlling bracken, i.e. 4-CPA, aminotriazole etc.; the toxic action of these is generally stated to be due to effects at the growing points - rhizome apices and developing buds - resulting in a decrease in frond numbers the following summer, and in some cases a degree of control in subsequent years (Jarvis, 1974). Their biochemical mechanisms of action in plants in general is covered in review publications such as Ashton and Crafts (1973) and Corbett (1974), although very little is known about their specific effects in bracken. More is known about the behaviour of asulam and glyphosate in bracken, probably as a result of their relative success. Asulam is taken up and translocated to the rhizome buds where distortion, lignification and fissuring of the tissues takes place (Veerasekaran et al., 1976; 1977). Williams and Foley (1975) found that while asulam had no effect on the storage carbohydrates after 20 months, glyphosate, in addition to its suppression of bud growth, reduced carbohydrate levels by approximately 50% and subsequent observations showed that the rhizomes decay more rapidly than with asulam (Williams, 1980). Such effects may contribute to glyphosate's slightly superior results to asulam in field control (Williams and Foley, 1975; Lea, 1977). The biochemical mechanism of action of glyphosate in plants in general is receiving a lot of attention. Inhibition of aromatic amino-acid biosynthesis has been proposed (Jaworski, 1972; Cole et al., 1980; Amrhein et al., 1980), as well as some starch grain disappearance

(Hull et al., 1977). Asulam is the only herbicide which shows some degree of selective activity in bracken control (Heywood, 1982), although some grass spp. are susceptible (Williams, 1977; Davies et al., 1979). Although asulam has been shown to effect some general biochemical processes in bracken buds, namely oxidative phosphorylation (Veerasekaran, 1975) and protein and RNA synthesis (Veerasekaran et al., 1976; 1977a), the biochemical basis for its selectivity is not known. Hence, a fuller study on its precise mechanism of action would be of value, not only for bracken but for its general use on weeds.

The erratic performance of foliar-applied herbicides and their failure to provide long-term control led workers to try soil applied herbicides for bracken control. The advantages here are obvious in that it is less hazardous to cover difficult terrain when the ground is visible and pre-emergence application can be carried out when farm labour is not occupied with more urgent operations (Fletcher, 1979). Several herbicides have some degree of control when used in this way. The most successful has been diclobenil. It has had most use in forestry situations (Spencer-Jones and Wilson, 1972) where its phytotoxicity to the indigenous grass sward is not so critical as in upland grazing pastures (Fletcher and Kirkwood, 1979). Picloram and dicamba have also shown reasonable control when applied before frond emergence, the degree of control being greater than their post-emergence activity (Lawson, 1964; Mitchell, 1968; Farnworth and Davies, 1974).

The herbicides which act on bracken through the soil have the major disadvantage for agricultural use of considerable persistence (Williams, 1980); a rapidly degradable herbicide with sufficient

mobility to make contact with the rhizome and buds could hence be desirable. Very little published information is available as to any pre-emergence activity on bracken by asulam. Williams (1980) states that asulam has very little soil activity against bracken. The reasons why this might be are not discussed.

1.5 Thesis objectives

The approach taken to improve bracken eradication with herbicides has tended to be a spray and watch type of programme which can only ever be of limited benefit. The approach adopted in this thesis was to carry out some fundamental studies, along the lines of those mentioned in 1.4 above, with the overall aim of improving the effectiveness of herbicides for bracken control.

Chapters 2 to 4 were directly concerned with these aspects. The principal herbicides involved here were asulam (methyl[4-amino-benzenesulphonyl]carbamate) and aminotriazole (3-amino-1,2,4-triazole). Chapters 5 and 6 involved a thorough study on the behaviour of iodide and thiocyanate salts when applied to plants. This aspect arose out of the work of chapter 4, where these two ions showed some interesting properties while acting as additives in formulations of bracken control chemicals.

Chapter 2: Studies on the mechanism of action of asulam.

This investigation set out to look at the mechanism of action of asulam in plants using in-vitro enzyme assay methods and plant growth studies. The aim was to gain information as to the reasons for asulam's success as a herbicide and its observed selective action as such.

Chapter 3: Studies on the influence of surfactants and other additives on the soil behaviour of asulam.

The purpose of this study was to investigate the possibility of application of asulam to bracken via the soil. Because of asulam's soil behaviour and the type of soils often found under bracken, some assistance from additives was envisaged in order to achieve this aim; laboratory experiments concerned with this were followed by a field trial.

Chapter 4: Ammonium thiocyanate and other compounds as additives in bracken control formulations.

The aim of this investigation was to develop a rapid assessment method for the effect of additives on the detoxification of aminotriazole and asulam by a free radical mechanism in bracken, with a view to optimising additive (especially ammonium thiocyanate) use and possibly developing new additives for bracken control formulations. The work involved mainly laboratory scorching bioassays prior to bracken control field trials.

Chapter 5: Studies on the selective herbicidal activity of iodide.

The aim of this investigation was to assess the role that endogenous thiocyanate levels played in the selective herbicidal action of iodide salts. Some biochemical and plant growth studies were employed.

Chapter 6: The mode of action of iodide and thiocyanate in plants.

This study was designed to investigate the mode of action of iodide and thiocyanate in plants and, especially, the variation within

the halides and the pseudohalide thiocyanate in their herbicidal and growth regulating effects on plants. The work again involved biochemical and plant growth studies.

Chapter 7: Conclusions.

The role of this chapter was to draw some conclusions as to the outcome of the studies and to make some suggestions for further investigations.

CHAPTER 2

STUDIES ON THE MECHANISM OF ACTION OF ASULAM

This chapter deals with aspects concerning the biochemical mode of action of asulam. The relevance of asulam as a foliar applied systemic herbicide for bracken control, the necessity for efficient uptake and translocation to the rhizome buds for activity and its partial selective action, has already been discussed (see chapter 1). Although this study does not involve bracken directly, the biochemical processes at the sites of action should be similar.

In previous work carried out in this laboratory, Babiker (1976) proposed a possible auxin involvement in asulam's mode of action. In following up his findings, preliminary investigations revealed an interesting interaction between PABA (p-aminobenzoic acid) and asulam. This interaction was considered very significant in terms of asulam's probable primary biochemical mechanism of action and so full attention was given over to this aspect. Once done, time did not permit the fuller scientific investigation of the forementioned auxin aspects. However, it is considered that a report of this work will be of value for any future investigation. Thus, a brief summary of this work is given below (2.A), prior to the main work of the chapter. Experimental details and results are given in Appendix 1.

2.A Preliminary investigations into asulam mode of action

Babiker (1976) has shown that asulam may possess auxin-type properties. He showed that, when applied to maize, asulam inhibited growth at high concentrations and stimulated it at low levels. These growth effects have been produced by IAA (indole-3-acetic acid) itself (Morré and Key, 1967) and other chemicals for whom auxin activity has been implied, including 2-4D (Hanson and Slife, 1969), picloram, (Eisinger and Morré, 1971) and the herbicidal triazines (Copping *et al.*, 1972). An increase in the *in-vitro* activity of IAA-oxidase by asulam and sulphanilamide, a major degradation product of asulam (Veerasekaran *et al.*, 1976), was also demonstrated (Babiker, 1976). The source of IAA-oxidase activity was horse-radish peroxidase (HRPO). Peroxidase (EC 1.11.1.7) preparations catalyse both IAA-oxidase and normal peroxidation reactions (Galston *et al.*, 1953; Saunders, 1964). A classical reaction of sulphanilamide is its ability to inhibit the oxidation of PABA by peroxidase/H₂O₂ systems (Lipmann, 1941). Babiker (1976) showed that sulphanilamide did indeed inhibit this reaction but asulam, a sulphonamide derivative (Cottrell and Heywood, 1965), stimulated the oxidation. Through the dual activity of plant peroxidase systems (peroxidase plus IAA-oxidase), some central role for PABA in asulam and sulphanilamide activity was envisaged.

Roots are extremely sensitive to auxins, growth being inhibited by concentrations as low as 10^{-7} - 10^{-8} M (Thimann, 1936). It is possible thus that any treatment which reduces or antagonises endogenous auxin (e.g. an increase in IAA-oxidase activity) might promote root growth (Robert *et al.*, 1976). Evidence for such a correlation has been

reported (Burström, 1950; Andreae and Collet, 1968). The initial preliminary experiment was to see how PABA affected IAA-oxidase activity. The results (Appendix A.1.1) showed that PABA stimulated IAA oxidation by HRPO, the increase in activity being greater than for asulam or sulphanilamide.

Studies involving the growth of wheat roots using a range of equimolar concentrations of asulam, PABA and sulphanilamide were carried out to compare relative toxicities and possible stimulatory activity. Asulam reduced ($P < 5\%$) wheat root lengths at all the levels tested, whereas PABA had no effect. In the case of sulphanilamide, the two highest concentrations reduced root growth. An increase in root length at the lowest sulphanilamide level was found to be non-significant (Appendix A.1.2). While asulam and sulphanilamide, at the levels employed, were still able to exert toxic effects, it was considered that, other things being equal, the greater ability of PABA to oxidise IAA may have relieved the roots from the inhibitory influence of endogenous IAA. Antagonism to root inhibition of exogenous IAA by PABA has been demonstrated (Audus and Quastel, 1948). Peroxidase action of the same enzyme may also have been responsible for insufficient PABA being available to further stimulate IAA-oxidase activity and result in root stimulation. To test some of these ideas, since sulphanilamide inhibits PABA peroxidation and asulam stimulates (Babiker, 1976), it was decided to combine lower, hopefully non-toxic, levels of these two compounds with PABA, in the hope that sulphanilamide would stimulate PABA root promotion and asulam would not. The levels employed, for asulam especially, were ill-chosen (Appendix A.1.3). For asulam alone,

36% root inhibition still resulted. Of considerable interest was that when combined with asulam, PABA completely overcame the inhibition. No effect from sulphanilamide alone, or in combination with PABA, resulted.

The interaction of asulam and PABA was considered significant in terms of the biochemical mode of action of asulam. All further work carried out on this aspect is reported below as the main work of this chapter.

2.1 INTRODUCTION

In addition to being a sulphonamide, asulam can also be regarded as a carbamic acid derivative (Cottrell and Heywood, 1965). The biochemical mode of action has been considered to closely resemble that of the N-phenylcarbamate herbicides (Cottrell and Heywood, 1965; Corbett, 1974). Veerasekaran (1975) demonstrated inhibition of oxidative phosphorylation while Veerasekaran et al. (1976, 1977a) have shown that it interferes with RNA and protein synthesis. Its morphological effects are also similar to those of the N-phenylcarbamates, causing stunted growth, chlorosis and eventual death by interfering with cell division and expansion in apical and axial meristems (Cottrell and Heywood, 1965). These symptoms result from applying the chemical to growing foliage or roots. Germinating seeds generally produce swollen and stunted roots and shoots and the plants fail to thrive (Ball et al., 1965). Sterrett and Fretz (1975) have reported arrested metaphases, anaphase and chromosome bridges in onion root-tip cells.

As stated previously, asulam is a sulphonamide derivative. The ability of PABA to prevent the inhibitory action of sulphonamides on micro-organisms is of course well documented (Woods, 1940; Shive, 1950). Various workers have shown that sulphanilamide will exert an inhibitory effect on root growth (Grace, 1938; Audus and Quastel, 1948). PABA has been shown to antagonise this inhibitory reaction (Mangenot and Carpentier, 1941; Bonner, 1942; Wielding, 1943; Brian, 1944; Audus and Quastel, 1948).

Taken together, these facts might suggest that as well as having carbamate and possible auxin properties, asulam may have the same phytotoxic action as the sulphonamides which were discarded as fungicides for use on plants due to this phytotoxicity (Rudd-Jones, 1956).

The initial aim of this study was to substantiate the aforementioned interaction between PABA and asulam on wheat growth. Wheat has been successfully used in similar sulphonamide studies (Brian, 1944). As the study progressed, a wider range of plants were tested. The specificity of the interaction was determined using compounds closely related to PABA. Furthermore, folic acid (pteroylglutamic acid), a major metabolic product of PABA (Iwai *et al.*, 1968), was also tested. Finally, some broader aspects of the interaction on wheat growth were studied.

2.2 EXPERIMENTAL

2.2.1 Materials

Asulam (99.5% purity) was purchased from the National Physical Laboratory. Sulphanilamide (May and Baker Ltd.) and all isomers of aminobenzoic acid (British Drug Houses Ltd.) were recrystallised from hot water (Perrlin et al., 1966). Folic acid was purchased from the Sigma Chemical Company, London. All other reagents were purchased from either British Drug Houses Ltd. or Hopkin and Williams Ltd., and were of the purest grades available.

2.2.2 Methods

2.2.2.1 Influence of PABA on the inhibition of wheat growth by asulam and sulphanilamide

Wheat (Triticum aestivum L. cv. Sicco) was sown into 100 ml beakers containing 9g of sieved vermiculite (2mm sieve) at a rate of 10 seeds/beaker. It was found necessary to sieve the vermiculite to remove the fines since variation between pots in water holding capacity was causing unacceptable variations in growth. The seeds were covered to a depth of 1 cm, treated with 50 ml of the appropriate solution and kept in a Fisons Growth Cabinet (Model 600 G3/THTL) at $25 \pm 0.75^{\circ}\text{C}$, $74 \pm 5\%$ r.h. and a 16h day length. The weights of the beakers were adjusted daily with deionised water. Seedling numbers were reduced to six/pot, normally the most evenly germinated. Root and shoot lengths and dry weights were ultimately measured 6-7 days after planting. Four beakers/treatment were employed.

Analysis of the data was carried out by one-way analysis of variance followed by LSD testing and Duncans Multiple Range test.

Two experiments were carried out; a) asulam (50 µg/beaker) and sulphanilamide (1875 µg/beaker) were assayed in the presence and absence of PABA (1500 µg/beaker); b) asulam (50 µg/beaker) was assayed in the presence and absence of a range of PABA concentrations (50-5000 µg/beaker) - a PABA control at 5000 µg/beaker was also included.

2.2.2.2 Influence of PABA on the inhibition of pea, bean and barley growth by asulam

Pea (Pisum sativum L. cv. Meteor) and dwarf French bean (Phaseolus vulgaris L. cv. Canadian Wonder) were sown in 400 ml beakers containing 36g of sieved vermiculite at a rate of six and four seeds/pot respectively. These were covered to a depth of 1 cm, treated with 200 ml of the appropriate solution and grown as previously described for wheat (2.2.2.1). Seedling numbers were reduced to four and three respectively per pot and measurements made 8 days after planting. Three beakers/treatment were employed. Barley (Hordeum vulgare L. cv. Midas) was grown as for wheat (2.2.2.1).

Analysis of the data was as previously described.

Four experiments were carried out; a) pea and bean were grown in the presence of a range of asulam concentrations (1000-10000 µg/beaker), b) pea and bean were grown in asulam concentrations (1000 and 2000 µg/beaker respectively) in the presence and absence of 6000 µg/beaker of PABA, c) bean was grown in asulam (2000 µg/beaker) in the presence and absence of 3000 µg/beaker of PABA, d) barley was grown in asulam (175 µg/beaker) in the presence and absence of PABA (1500 µg/beaker).

2.2.2.3 Influence of compounds structurally related to PABA on the inhibition of wheat growth by asulam

Wheat was grown and the resulting data treated as in 2.2.2.1. Three experiments were carried out; a) wheat was grown in the presence of a range of *o*-aminobenzoic acid (OABA) and *m*-aminobenzoic acid (MABA) concentrations (50-5000 µg/beaker), b) comparison of the influence of OABA, MABA and PABA (1500 µg/beaker) on asulam (50 µg/beaker) inhibition of growth was made, c) PABA (1500 µg/beaker) and equimolar concentrations of *p*-hydroxybenzoic acid, *p*-chlorobenzoic acid, *p*-chloroaniline and *p*-aminophenol were assayed in the presence and absence of asulam (250 µg/beaker).

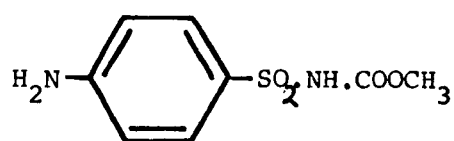
Structures of the compounds used in this section are given in figure 2.1.

2.2.2.4 Influence of folic acid on asulam inhibition of wheat and barley growth

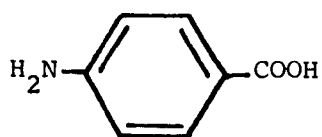
Wheat and barley were grown and assessed as per 2.2.2.1. For wheat, folic acid (1000 and 2500 µg/beaker) was assayed in the presence and absence of asulam (200 µg/beaker). In the barley experiment, 500 and 2500 µg/beaker of folic acid was employed. To facilitate dissolution, all folic acid solutions were prepared in 10^{-3} M NaHCO₃. Appropriate controls showed no effect in growth due to this weak base.

2.2.2.5 Influence of PABA on asulam inhibition of soil grown wheat

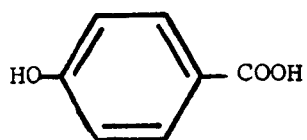
Wheat was sown in 100 ml beakers containing 35g of sieved (2mm) fresh soil at the rate of 10 seeds/beaker. The soil used was the B₃ horizon of the Carbeth soil (see chapter 3). The seeds were covered by a further 5g of soil and treated with 2 ml of the appropriate solution.



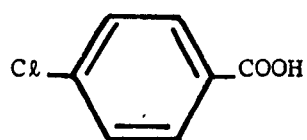
asulam



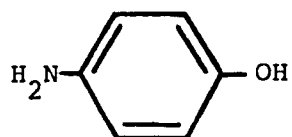
PABA



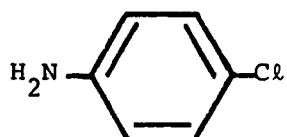
p-hydroxybenzoic acid



p-chlorobenzoic acid



p-aminophenol



p-chloroaniline

Figure 2.1 Structures of PABA and related compounds assessed for antagonistic activity against asulam wheat root inhibition.

The beakers were then kept and assessed as above (2.2.2.1). Asulam (275 μg , equivalent to approximately 7 $\mu\text{g/g}$ of fresh soil) was applied in the presence and absence of 6000 μg of PABA (150 $\mu\text{g/g}$).

2.2.2.6 Influence of PABA on asulam inhibition when the two are not applied simultaneously

With some modifications, wheat was grown and assessed as per 2.2.2.1. Two experiments were carried out: a) Wheat treated with deionised water alone was grown for 4 days and then treated with 375 μg /beaker of asulam. This was compared to wheat grown initially in 1500 μg /beaker of PABA before asulam addition. Eight beakers/treatment were employed. In half the pots, the wheat was transplanted into fresh vermiculite before the addition of asulam, whilst for the other half, the plants were left in their original growing medium. Assessment was made after a further 7 days; b) wheat was grown in the presence of 375 μg /beaker of asulam for 4 days before the addition of 1500 μg of PABA or deionised water. Four pots/treatment were employed.

2.2.2.7 Influence of PABA on asulam inhibition of wheat growth when both compounds are foliar applied

Wheat was sown on 28/5/79 into 6 cm plant pots containing compost (Fisons Levington). The seeds (12/pot) were covered to a depth of 1 cm before the addition of 25 ml of water. The pots were kept at a sheltered spot on the laboratory roof and watered when there had been insufficient rainfall to keep the compost reasonably moist. Plant numbers were reduced to the six most even seedlings/pot between 60 and 70 mm high (1st leaf stage) before spraying with the appropriate

treatments on 16/6/79. Spraying was carried out using a Boots hand sprayer set under pre-determined conditions to give a known spray volume per unit area. Asulam was applied as Asulox (May and Baker Ltd.) at a rate equivalent to 2 kg/ha. In order to dissolve sufficient PABA, the Na salt was prepared using NaOH. Na-PABA was applied at a rate equivalent to 15 kg/ha PABA in the presence and absence of Asulox. All the spray solutions were made up in 0.2M pH 7 phosphate buffer (due to difficulties encountered in stabilising Na-PABA preparations at a neutral pH) containing 200 µl/l of the commercial surfactant Agral (ICI Ltd.). The sprayed pots were held in a growth cabinet (see 2.2.2.1) at $15 \pm 0.75^{\circ}\text{C}$, 100% r.h. overnight to facilitate maximum uptake, before being returned to the roof. Assessment was made on 23/7/79.

2.3 RESULTS

Unless otherwise stated, only root and shoot lengths, which are expressed as percentages of their control lengths, are presented here. Although root and shoot dry weights were also determined, they were found to have identical trends to the length measurements and are omitted for reasons of clarity. Over the short time period of the experiments, shoot growth was never inhibited to the same extent as root growth and hence emphasis is placed on reporting the latter data.

Levels of asulam or sulphanilamide were generally chosen which would result in approximately 50% inhibition of root length. Differences at $P < 5\%$ were taken as being significant.

2.3.1 Influence of PABA on the inhibition of wheat growth by asulam and sulphanilamide

As found in the preliminary experiments (Appendix A.1.3), when asulam (50 $\mu\text{g}/\text{beaker}$) was assayed in the presence of PABA (1500 $\mu\text{g}/\text{beaker}$) its root inhibition was completely overcome. When sulphanilamide was employed at a toxic level (1875 $\mu\text{g}/\text{beaker}$), its toxicity was also antagonised (table 2.1).

Table 2.1 Influence of PABA on the inhibition of wheat growth by asulam and sulphanilamide

Treatment	Mean shoot length	Mean root length
control	a 100 (5)	a 100 (4)
asulam (50)	a 103 (12)	b 61 (10)
sulphanilamide (1875)	b 86 (2)	b 47 (3)
PABA (1500)	a 110 (3)	a 91 (13)
asulam (50)+PABA (1500)	a 106 (4)	a 102 (6)
sulphanilamide (1875)+PABA (1500)	a 107 (6)	a 98 (5)

Treatment concentrations are in $\mu\text{g}/\text{beaker}$. Assessment was made after 6 days. Shoot and root lengths are expressed as percentages of the control lengths. Figures in parentheses (length data) are standard deviations (n-1 D.F.).

Values with a similar letter, within each column, are not significantly different at the 5% level in Duncans Multiple Range test.

When a range of PABA concentrations (50–500 µg/beaker) were combined with asulam (50 µg/beaker), the inhibitory effect of asulam was reduced at all levels. These levels correspond to PABA:asulam molar ratios of between 167:1 and 2:1. At two levels of PABA (1500 and 750 µg/beaker, PABA:asulam 50:1 and 25:1), the effects of asulam were completely overcome. At the highest level tested (5000 µg/beaker), PABA inhibited root growth (table 2.2). Levels up to 1500 µg/beaker had already been shown to have no effect on growth (Appendix A.1.2).

Table 2.2 Influence of a range of PABA concentrations on the inhibition of wheat growth by asulam

Treatment	Mean shoot length	Mean root length
control	a 100 (12)	a 100 (11)
asulam (50)	a 100 (10)	e 52 (4)
PABA (5000)	a 93 (14)	cb 77 (6)
asulam(50)+PABA(5000)	a 103 (1)	b 85 (4)
asulam(50)+PABA(1500)	a 96 (7)	a 94 (5)
asulam(50)+PABA(750)	a 105 (15)	a 100 (7)
asulam(50)+PABA(300)	a 106 (6)	cb 83 (8)
asulam(50)+PABA(150)	a 100 (8)	dc 74 (1)
asulam(50)+PABA(50)	a 103 (6)	d 65 (5)

All details are as for table 2.1

2.3.2 Influence of PABA on the inhibition of pea, bean and barley by asulam

Experiments were carried out to check that the PABA antagonism of asulam occurred in plants other than wheat.

The effects of asulam on the growth of pea and bean are shown in table 2.3.

Table 2.3 Influence of asulam on the growth of pea and bean

Treatment	Mean shoot length	Mean root length
a) <u>Pea</u>		
control	a 100 (5)	a 100 (3)
asulam (1000)	b 89 (5)	b 53 (15)
asulam (5000)	c 36 (19)	c 22 (22)
asulam (10000)	d 5 (6)	c 15 (3)
b) <u>Bean</u>		
control	a 100 (13)	a 100 (6)
asulam (1000)	a 90 (6)	b 84 (7)
asulam (5000)	b 64 (3)	c 52 (2)
asulam (10000)	b 60 (4)	c 49 (3)

Assessment was made after 8 days. All other details as for table 2.1.

All levels (1000-10000 $\mu\text{g}/\text{beaker}$) reduced root growth although pea appeared more susceptible than bean. PABA (6000 $\mu\text{g}/\text{beaker}$) reduced inhibition of pea roots (table 2.4a).

Table 2.4 Influence of PABA on the inhibition of pea, bean and barley growth by asulam

Treatment	Mean shoot length	Mean root length
a) <u>Pea</u>		
control	a 100 (3)	a 100 (12)
asulam(1000)	a 93 (1)	b 56 (3)
PABA(6000)	a 110 (11)	a 103 (11)
asulam(1000)+PABA(6000)	a 102 (8)	a 109 (7)
b) <u>Bean</u>		
(i) control	a 100 (3)	a 100 (6)
asulam(2000)	c 71 (5)	b 63 (6)
PABA(6000)	b 86 (4)	b 71 (10)
asulam(2000)+PABA(6000)	ba 93 (3)	b 73 (3)
(ii) control	a 100 (5)	a 100 (5)
asulam(2000)	b 82 (1)	c 55 (3)
PABA(3000)	a 103 (5)	a 93 (5)
asulam(2000)+PABA(3000)	a 100 (3)	b 74 (2)
c) <u>Barley</u>		
control	a 100 (4)	a 100 (5)
asulam(175)	c 67 (4)	c 36 (11)
PABA(1500)	b 82 (5)	ba 93 (10)
asulam(175)+PABA(1500)	b 84 (5)	b 86 (3)

Bean and pea were assessed 8 days after treatment, barley 7 days.
All other details as for table 2.1.

Bean was more susceptible than pea to PABA toxicity and thus it was found necessary to use a lower level of PABA (3000 $\mu\text{g}/\text{beaker}$) before a significant inhibition of asulam (2000 $\mu\text{g}/\text{beaker}$) effect could be detected (2.4b). PABA (1500 $\mu\text{g}/\text{beaker}$) overcame the asulam (175 $\mu\text{g}/\text{beaker}$) inhibition of barley root growth (table 2.4c).

2.3.3 Influence of compounds structurally related to PABA on the inhibition of wheat growth by asulam

Experiments were carried out to check the specificity of PABA in antagonising asulam inhibition of wheat growth.

At concentrations up to 5000 $\mu\text{g}/\text{beaker}$, OABA and MABA had no significant effect on wheat root growth. Shoots were inhibited at the highest MABA level tested (table 2.5a). Combinations of these chemicals with asulam, at levels equivalent to the optimum PABA/asulam combination for maximum antagonism (1500 and 50 $\mu\text{g}/\text{beaker}$, PABA and asulam, respectively) revealed that neither compounds reduced the effect of asulam. Again, PABA completely overcame the effect of asulam (table 2.5b).

At concentrations equivalent to 1500 $\mu\text{g}/\text{beaker}$ of PABA, none of the other related compounds tested showed PABA-type effects on asulam (250 $\mu\text{g}/\text{beaker}$) inhibition. *p*-Chloroaniline brought about some inhibition of growth on its own and its combination with asulam was subsequently found to be more effective in reducing growth than asulam alone (table 2.6).

Table 2.5 Influence of OABA and MABA on wheat growth and their interaction with asulam

Treatment	Mean shoot length	Mean root length
a) control	^a 100 (5)	^a 100 (8)
OABA (50)	^a 99 (3)	^a 90 (17)
OABA (150)	^a 102 (4)	^a 105 (17)
OABA (1500)	^a 93 (9)	^a 85 (11)
OABA (5000)	^a 90 (7)	^a 73 (18)
MABA (50)	^a 94 (8)	^a 98 (18)
MABA (150)	^a 98 (14)	^a 86 (19)
MABA (1500)	^a 97 (6)	^a 85 (8)
MABA (5000)	^b 77 (11)	^a 70 (8)
b) control	^a 100 (5)	^a 100 (6)
asulam (50)	^a 93 (10)	^b 62 (8)
asulam (50)+OABA (1500)	^a 99 (7)	^b 54 (4)
asulam (50)+MABA (1500)	^a 99 (8)	^b 54 (2)
asulam (50)+PABA (1500)	^a 106 (3)	^a 101 (5)

All details are as for table 2.1

Table 2.6 Influence of PABA and equimolar concentrations of several structurally related compounds on the inhibition of wheat growth by asulam

Treatment	Mean shoot length	Mean root length
control	a 100 (6)	a 100 (6)
asulam(250)	b 76 (12)	d 37 (3)
PABA(1500)	a 97 (5)	a 99 (6)
p-hydroxybenzoic acid(1510)	a 99 (8)	ba 97 (6)
p-chlorobenzoic acid(1713)	a 100 (8)	a 100 (3)
p-chloroaniline(1395)	b 70 (8)	c 79 (1)
p-aminophenol(1193)	a 91 (7)	ba 95 (3)
asulam(250)+p-hydrobenzoic acid(1510)	b 66 (12)	d 37 (4)
asulam(250)+p-chlorobenzoic acid(1713)	b 76 (2)	d 39 (4)
asulam(250)+p-chloroaniline(1395)	c 36 (13)	e 27 (2)
asulam(250)+p-aminophenol(1193)	b 77 (9)	d 41 (5)
asulam(250)+PABA(1500)	a 95 (4)	b 91 (2)

All details are as for table 2.1

2.3.4 Influence of folic acid on asulam inhibition of wheat and barley growth

The important PABA metabolite, folic acid, was toxic to wheat roots at the two levels employed (1000 and 2500 $\mu\text{g}/\text{beaker}$). Equimolar concentrations of PABA (i.e. 311 and 777 $\mu\text{g}/\text{beaker}$) would not be toxic in such a situation. When these levels of folic acid were applied with asulam (200 $\mu\text{g}/\text{beaker}$), reduction in the growth inhibition by asulam resulted (table 2.7a).

No reduction in growth was observed when folic acid was applied to barley (500-2500 $\mu\text{g}/\text{beaker}$). These levels antagonised the inhibition of root growth in barley by asulam (200 $\mu\text{g}/\text{beaker}$) (table 2.7b).

2.3.5 Influence of PABA on asulam inhibition of soil grown wheat

It was decided to see if the PABA antagonism of asulam in wheat could be extended to situations other than the germination and early growth in vermiculite pot experiments.

The first such experiment shows that the antagonism will occur when the wheat is grown in fresh soil. 83% root inhibition in the presence of asulam (300 $\mu\text{g}/\text{beaker}$) alone was reduced to 15% in the presence of PABA (6000 $\mu\text{g}/\text{beaker}$) (table 2.8).

Table 2.7 Influence of folic acid on the inhibition of wheat and barley growth by asulam

Treatment	Mean shoot length	Mean root length
a) <u>Wheat</u>		
control	a 100 (5)	a 100 (5)
asulam(200)	b 76 (11)	e 42 (2)
folic acid(1000)	a 99 (5)	b 83 (4)
folic acid(2500)	a 100 (4)	c 67 (3)
asulam(200)+folic acid(1000)	a 100 (6)	d 53 (5)
asulam(200)+folic acid(2500)	a 98 (4)	c 62 (2)
b) <u>Barley</u>		
control	a 100 (5)	a 100 (6)
asulam(200)	a 93 (3)	e 63 (3)
folic acid(500)	a 95 (3)	a 100 (4)
folic acid(1500)	a 99 (3)	a 106 (10)
folic acid(2500)	a 97 (2)	ba 98 (6)
asulam(200)+folic acid(500)	a 97 (3)	d 80 (4)
asulam(200)+folic acid(1500)	a 96 (3)	cb 91 (5)
asulam(200)+folic acid(2500)	a 98 (1)	c 89 (3)

Wheat was assessed after 6 days, barley after 7 days.

All other details as for table 2.1.

Table 2.8 Influence of PABA on asulam inhibition of soil grown wheat

Treatment	Mean shoot length	Mean root length
control	a 100 (11)	a 100 (5)
asulam(300)	b 22 (7)	c 17 (8)
PABA(6000)	a 102 (8)	a 101 (5)
asulam(300)+PABA(6000)	a 100 (11)	b 85 (3)

All details as for table 2.1.

2.3.6 Influence of PABA on asulam inhibitions of wheat when the two are not applied simultaneously

Experiments were carried out to investigate the possibility of bolstering PABA levels in plants to tolerate later asulam additions or to release plants from asulam injury with PABA treatment.

Asulam (375 µg/beaker) was toxic to 4 day old seedlings. When the seedlings had been initially grown in the presence of 1500 µg/beaker of PABA, the direct addition of asulam resulted in significantly less asulam toxicity. Since there may have been PABA still in the rooting medium at four days, it was decided to transplant some seedlings into fresh vermiculite before the addition of asulam. Under these conditions, asulam was toxic to previously water grown wheat, however, although PABA grown wheat was inhibited to a lesser degree, the difference was not significant (table 2.9).

Table 2.9 Influence of the pre-addition of PABA to asulam inhibition of wheat

Treatment	Mean shoot length	Mean root length
control ⁺	a 100 (6)	a 100 (4)
water day 0, asulam(375)day 4 ⁺	a 89 (5)	c 67 (2)
PABA(1500)day 0, asulam(375)day 4 ⁺	a 91 (7)	b 80 (7)
control [*]	a 93 (2)	b 86 (2)
water day 0, asulam(375)day 4 [*]	a 92 (2)	d 57 (7)
PABA(1500)day 0, asulam(375)day 4 [*]	a 88 (9)	d 60 (2)

⁺ not transplanted day 4

^{*} transplanted into fresh vermiculite day 4

Assessment was made after 11 days.

All other details as table 2.1.

When wheat was grown in the presence of asulam (375 µg/beaker) for 4 days before the direct addition of 1500 µg of PABA to the rooting medium, slightly less eventual (11 days) toxicity resulted compared to that with no PABA addition. Again, this difference was not significant (table 2.10).

Table 2.10 Influence of PABA on wheat previously inhibited by asulam

Treatment	Mean shoot length	Mean root length
asulam(375)day 0, water day 4	^a 100(4)	^a 100(21)
asulam(375)day 0, PABA(1500)day 4	^a 109(5)	^a 137(12)

Assessment made after 11 days. Root and shoot lengths are percentages of the asulam control lengths.

All other details as for table 2.1.

2.3.7 Influence of PABA on asulam inhibition of wheat growth when both compounds are foliar applied

This experiment was conducted to investigate if the antagonism of asulam by PABA takes place when they are applied through the foliage to growing wheat plants. In this comparatively longer term experiment, the presence of large matted root systems made measurement of root lengths impractical. Thus, shoot length and shoot and root dry weights are presented (table 2.11).

Foliar applied asulam (2kg/ha) markedly reduced root weight compared to the control. Less inhibition resulted when PABA (15kg/ha) was also applied. Foliage dry weight measurements also followed these trends. The application of PABA alone significantly increased shoot length. Although an increase in dry matter yield with PABA occurred, this difference was non-significant ($P > 5\%$). No decrease in tiller

Table 2.11 Influence of PABA on asulam inhibition of wheat growth
when both compounds are foliar applied

Treatment	Mean shoot length	Mean root weight(g)	Mean shoot weight (g)
control	^b 100	^a 0.64	^a 0.73
asulam(2)	^c 60	^c 0.12	^c 0.10
PABA(15)	^a 112	^a 0.59	^a 0.86
asulam(2)+PABA(15)	^c 62	^b 0.26	^b 0.30

Treatment levels are in kg/ha. Asulam applied as "Asulox", PABA as the Na-PABA salt. Assessment made 34 days after spraying.

All other details as for table 2.1.

number or growth was observed when sampling was carried out. Shoot lengths of asulam treated plants were unaffected by the simultaneous application of PABA although an increase in dry matter yield occurred. Less tillers in asulam alone treated plants compared to those which received the combination was observed.

2.4 DISCUSSION

The results of this investigation showed that PABA can reduce, and in some cases completely overcome, the toxicity of asulam to a range of plant species grown in vermiculite. By assaying a range of related compounds it was shown that this action is specific to PABA and its metabolic product folic acid. Furthermore, the activity of PABA was found to extend to wheat sown in fresh soil and when asulam and PABA were foliar applied to growing wheat.

The PABA:asulam ratio required to completely overcome the effects of asulam in wheat was 25:1 or perhaps slightly less. Audus and Quastel (1948) demonstrated a similar effect with sulphanilamide where only a 1:1 ratio was required. However, as can be seen from Appendix A.1.2, asulam is a considerably more toxic compound. Although there is an obvious parallel here with the PABA reversal of sulphanilamide toxicity to micro-organisms, Audus and Quastel (1948) suggested that this type of specific interaction is not involved in plants as much greater concentrations of PABA relative to sulphanilamide are required. However, they did not propose any possible reaction mechanism. Brian (1944), on the other hand, suggests that the interaction occurring in micro-organisms is common to several widely distinct types of living organisms, including plants. Slow movement through tissues (Rudd-Jones, 1956) and rapid conjugate formation (Shive, 1950) could partly account for high concentrations of PABA being necessary in plants.

After much other evidence had been published, Angier et al. (1946) finally established that in micro-organisms, sulphanilamide acted by preventing the synthesis of folic acid from PABA. More recent work on plants (Iwai et al., 1968), using cell free extracts from pea seedlings, has established the presence of an enzyme, 7,8-dihydrofolate synthetase (EC 1.5.1.3), which can catalyse the formation of folic acid from PABA. Thus, it is possible that asulam may be competing in folic acid synthesis. Since this acid is one of the factors involved in biological methylations, failure or impairment of these one-carbon transfers could result in reduced formation of a range of amino acids and the purine bases which would in turn reduce protein and nucleic acid synthesis. Such a theory would not be inconsistent with the work of Veerasekaran et al. (1976, 1977a). The fact that PABA can be replaced by folic acid in these experiments supports this argument, although the possibility of breakdown to PABA within the plant cannot be excluded. The possibility of differential uptake also exists, PABA blocking the site of asulam uptake at the root surface. When folic acid was used, only trace amounts of PABA could be detected in the solution surrounding the roots. However, identical quantities were also found in freshly prepared folic acid solutions. This would suggest that the method of detection (Bratton and Marshall, 1939) was bringing about slight hydrolysis of folic acid to PABA. Even if this were not the case, the quantities of PABA involved were of the order of less than 1.0 μg of PABA in 50 μg of folic acid. This would be insufficient to bring about the reversal of asulam toxicity. Additionally, it would seem unlikely that this blocking would be of such a specific nature. Audus and

Quastel (1948), using cress, have shown that root inhibition by exogenous IAA was antagonised by PABA. This may be due to a stimulation of IAA-oxidase activity. The levels of PABA required to overcome the effect of IAA were high (10000:1). In conjunction with their findings for the ratios with PABA to overcome sulphanilamide activity (1:1), they concluded that the phenomenon of root growth inhibition by sulphanilamide was quite distinct from that by plant growth substances. Similar conclusions could be drawn from the behaviour of asulam (25:1).

It is somewhat surprising that no reported evidence existed in the literature of the PABA/asulam interaction, bearing in mind the well-documented PABA/sulphonamide studies in micro-organisms and plants (Audus and Quastel, 1948 ; Shive, 1950; Rudd-Jones, 1956). In the plant studies, Audus and Quastel (1948) were investigating possible hormonal properties, while the work of Rudd-Jones (1956) was primarily to confer bacteriocidal rather than phytotoxic properties in treated plants; the fact that PABA could on occasion overcome the latter's effect was nullified by a coincident loss of bacteriocidal properties. However, for asulam, the principal, if not the only aim, is to eradicate plants, preferably in a selective manner.

Evidence from the literature (Lewis, 1942; Thompson et al., 1943; Sebrell and Harris, 1954) and from some preliminary experiments conducted in this laboratory (Kidd, 1982) would suggest that variations exist between plants in endogenous levels of PABA. The reason for these differences could be due partly to species differences, but environmental conditions or stage of growth are also likely to be involved. Bean seedlings are reported as having a higher PABA content than many other

seedlings at a comparable stage of growth (Barton and MacNab, 1956). As beans are more resistant to asulam than the other species tested, it would be tempting to account in part, at least, for the resistance in terms of endogenous PABA levels. On the other hand, the level of folates present in plants may be the critical factor in determining resistance. In the present study, pre-addition, to bolster the PABA levels in wheat, was successful to a limited extent in that tolerance to asulam was slightly enhanced. Attempts to improve the results here were not successful. If any less asulam was used, non-significant root inhibition occurred, additionally, if higher PABA levels were employed, its toxicity increased, thus masking any beneficial effects.

The findings of this study may provide potential for the widening of the selectivity of asulam in weed control. To achieve this, a good deal of further work would be necessary on aspects such as the movement and metabolism of PABA in plants and in the determination of optimum PABA:asulam ratios in a wide range of species. As found in the present study for bean, tolerance of the weed or crop species to PABA could be crucial, e.g. in crops susceptible to asulam, if they are tolerant to PABA then selective control over more PABA-susceptible weeds may be achieved. The fact that the antagonism by PABA results from both root and foliar application may aid in such studies.

These results do not preclude other roles for PABA in plants. Remarkably little information is available on this point. Mangenot and Carpentier (1941a), using pea roots, showed that lateral root stimulation by exogenous IAA was augmented by PABA. Stimulation of shoot growth was observed for PABA in the present study (table 2.11). The reasons for

this are unclear. The stimulation of plant growth by sub-toxic applications of herbicides and other compounds is common and explanation of the general or individual mechanism of action are sparse (Ries, 1976). Folate levels may be a factor partially limiting growth which PABA could be expected to enhance. Additionally, activation of enzymes, including IAA-oxidase (see Appendix A.1.1), may be occurring. Pilet (1966), using the related compound p-hydroxybenzoic acid, inversely correlated in-vitro IAA-oxidase activity with endogenous auxin content and growth of excised lentil stems. At low concentrations, positive growth effects on several unspecified biochemical processes were found (Pilet, 1966).

The results of the present study also do not exclude contributions from carbamate or auxin-type activities to the total toxic effect produced by asulam. The specific and total nature of the PABA/folate antagonism does tend to suggest that the sulphonamide activity is the primary mechanism involved. It should be remembered that herbicides applied in one molecular form may require to be metabolised to a different structure to express their inhibition (Hilton et al., 1963). The asulam metabolite sulphanilamide (Veerasekaran et al., 1976) may be the toxic agent, the apparent greater toxicity of asulam versus sulphanilamide (see table 2.1 and appendix A.1.2) being due to, perhaps, effectively greater accumulation near the active site, before its hydrolysis to the toxic agent.

CHAPTER 3

STUDIES ON THE INFLUENCE OF SURFACTANTS AND OTHER ADDITIVES ON THE SOIL BEHAVIOUR OF ASULAM

3.1 INTRODUCTION

Some advantages that pre-emergence soil applied treatments may have over foliar spraying for bracken control and the possibility of using asulam in such a role were introduced in chapter 1. When considering such an approach, appreciation of the effects on persistence and availability of the herbicide at the site of uptake by its interaction with soil, plant and environmental factors, need also be considered (Babiker, 1976). There is little published information on asulam being applied to the soil for bracken control. From the discussions of chapter 1, it is obvious that to be successful, the asulam would have to make contact with the developing and dormant buds of the rhizome system. It may well be that difficulties encountered reaching this system may account for its apparent low soil activity as reported by Williams (1980).

The main factors which modify the intrinsic activity of a soil applied herbicide are: adsorption of the chemical by soil; its vertical distribution or leaching; its persistence in an active form; and the soil type encountered. These factors are complex and inter-related and the reader is referred elsewhere to some excellent reviews for more comprehensive coverage than will be given here - see Holly (1961); Upchurch (1966); Bailey and White (1970); Babiker (1976) and Hance

(1980). Of the factors affecting availability of soil applied chemicals to the target plant, soil degradation and adsorption are generally considered to be the most important (Holly, 1961; Upchurch, 1966; Bailey and White, 1970). Herbicides in soil can be inactivated by biological or non-biological degradation (chemical, photochemical and loss by volatilisation), the former being by far the most important (Holly, 1961). As well as restricting the quantity of chemical available for plant uptake, adsorption is very important in determining the ease with which herbicides move through the soil profile away from or to the zone of action (Upchurch, 1966). Water solubility of the chemical and post-application rainfall will also be important factors in determining the extent of leaching (Upchurch, 1966). The most important soil properties affecting adsorption and degradation are organic matter content, pH, texture, structure and moisture content. The organic matter content is generally considered the most important soil property affecting adsorption of herbicides on soils (Hamaker and Thompson, 1972 ; Weed and Weber, 1974; Babiker, 1976), this property being highly correlated with adsorption for nearly every chemical (Hamaker and Thompson, 1972). Degradation is often significantly correlated with organic matter content, however, high correlations to other, often inter-related, soil properties (cation-exchange capacity, texture), as well as to adsorption, makes it difficult to determine its individual effect on rate of herbicide loss (Hamaker, 1972; Walker, 1977). pH also is often important in adsorption and leaching patterns, especially for herbicides capable of taking on a charge; the effect of pH is well illustrated by its influence on asulam-soil behaviour.

Asulam is very mobile in soils (Anon, 1971; Babiker and Duncan, 1975a), its movement being influenced by pH where undissociated asulam ($pK_a = 4.8$) leaches less rapidly than the ionic species (Babiker and Duncan, 1975a). Soil adsorption of asulam is generally low (Babiker and Duncan, 1975a; 1977; Smith and Walker, 1977). The adsorption that does occur has been inversely correlated with pH, probably due to change in the net charge of the molecule where its affinity towards the adsorbents present changes (Babiker and Duncan, 1977). Asulam adsorption is also highly correlated with organic matter and to a lesser extent with cation exchange capacity (Babiker and Duncan, 1977). Organic matter is a major adsorbent for undissociated pesticide species (Hartley, 1976). Carringer *et al.*, (1975) found that asulam adsorption by organic matter was in the reverse order of its solubility and suggested that the mechanism of adsorption was hydrophobic bonding. Asulam is not adsorbed onto clay to any great extent. Babiker and Duncan (1977) found no correlation between adsorption and clay content while Carringer *et al.*, (1975) found that calcium montmorillonite at pH 6 adsorbed no asulam. Babiker (1976) also observed negligible adsorption with montmorillonite at neutral pH's, as would be expected from the repulsion of asulam anion by the negatively charged clay particles. Slight positive adsorption at pH 3.2 (Babiker, 1976) was probably due to protonation of the amino-nitrogen by acidic water co-ordinated to the interlayer cation of the clay (Fusi *et al.*, 1980; Ristori *et al.*, 1981). The correlation of adsorption with cation exchange capacity found by Babiker and Duncan (1977), is probably due to the large contribution made to that phenomenon by organic matter (Russell, 1973).

Soil degradation of asulam is generally rapid. High rates of disappearance have been found under conditions conducive to high rates of microbial activity, i.e., high organic matter, high temperature and high moisture content (Anon, 1971; Babiker and Duncan, 1975a; 1977; Smith and Walker, 1977), indicating that the pathway of degradation is probably mainly biological. Higher rates of degradation were found in top-soil than in sub-soils, for the same reasons (Babiker and Duncan, 1977).

The soils under bracken are typically friable and porous, with a surface layer often high in organic matter (Mitchell, 1973). A wide range in pH values are encountered in bracken soils (3.6 to 7.6, mean 5.5 - ref. Salisbury, 1925). From the foregoing discussion, these properties will present special problems to the use of pre-emergence soil applied asulam for bracken control, resulting in rapid degradation and difficulties in the placement of the chemical within the soil profile: In highly organic alkaline soils, low adsorption (hence high leaching) and rapid degradation would be expected - a high degree of leaching may result in loss from the horizon altogether and could cause environmental problems (Babiker and Duncan, 1975a); in highly organic acid soils, greater adsorption (less leaching) accompanied by rapid degradation would be expected - the increase in adsorption could be significant, depending on the organic matter content and degree of soil acidity encountered. More fixation near the surface may enhance the quantity available to be inactivated by degradation (Babiker and Duncan, 1977).

The pre-emergence use of asulam in other agricultural situations has resulted in conflicting reports as to its effective use.

Menges et al., (1972) showed prolonged pre-emergence weed control in watermelon on loamy sand soils (pH 6-8, organic matter 0.7%-0.8%) under furrow irrigation. Brock (1972) found only short term pre-emergence activity on docks on a sandy loam soil (no details), while Ball et al., (1965) claimed long term pre-emergence control over a wide range of weed species on a range of soils. Interpretation of these inconsistencies have been made by Babiker and Duncan (1977), based very much on the soil behaviour of asulam as discussed above.

The success of asulam as a pre-emergence herbicide will be determined by its ability to function over a wide range of conditions with adequate reliability. The effective and safe use for bracken control, or in other weed control situations, could be achieved, or at least improved, if some degree of control over its behaviour in soil could be accomplished. If the adsorption, and thus its movement or position in the soil, could be regulated within precise limits, its usefulness in a pre-emergence situation could be increased. Equally, control over its degradation would be advantageous, in terms of both increasing and decreasing persistence, depending on the circumstances. A look at the literature revealed two interesting approaches to the manipulation of herbicide behaviour in soil.

1. The addition of surfactants has been used to alter the adsorption, movement and degradation of pesticides in soil. Several studies have shown that surfactants, either through their effect on soil physical properties affecting water infiltration rates or on pesticide adsorption, can regulate pesticide movement under laboratory conditions. Bayer (1967) reported diverse effects of surfactants on the movement of

substituted ureas, the surfactants employed causing both increases and decreases in herbicide leaching. Smith and Bayer (1967) showed that the effects of surfactants on the adsorption of diuron was inversely related to their effects on the rate of leaching. Several cationic surfactants increased diuron adsorption while the non-ionic surfactants tested had no effect (Smith and Bayer, 1967). Koren (1972) achieved enhanced movement of trifluralin into soils, resulting in an increase in activity of the herbicide, which normally requires mechanical incorporation into soils to prevent loss by volatilisation (Savage and Barrentine, 1969). Huggenberger et al., (1973) found that adsorption of lindane, diuron and atrazine by soil from a surfactant solution was inversely related to the leaching of the herbicides through soil columns in the presence of the surfactants. Of particular interest is the effects by surfactants found by Gaynor and Volk (1976) on picloram adsorption by nine Oregon soils. They found that the anionic surfactant, sodium dodecylbenzene sulphonate markedly decreased picloram adsorption on all nine soils at a 1% w/v solution level. The cationic detergent used, cetyl pyridinium chloride, significantly increased adsorption on seven of the soils at a similar level, virtually eliminating the effects of pH and organic matter on picloram adsorption in two of these soils. Like asulam, picloram is an acidic herbicide; its pKa being 3.4 (Cheung and Biggar, 1974). It has been applied pre-emergence to bracken, achieving good short term control (Lawson, 1964); Farnworth and Davies, 1974).

Field performance of soil applications of the fungicide benomyl, for the control of Verticillium wilt in cotton (Rawlings and Booth, 1968) and potato (Biehn, 1970), has been improved by the addition

of surfactants to enhance the movement into the soil without physical incorporation.

Increased soil persistence of pesticides has also been accomplished with the addition of surfactants. Danielson et al., (1961) showed that low levels of non-ionic and cationic detergents increased the persistence of EPTC in soils. The addition of anionic detergents to soil increased the persistence of the organophosphate insecticides diazinon and parathion. Two months after application to a silty loam soil, thirteen times more parathion and five times more diazinon were present over controls when a linear alkyl benzene sulphonate detergent had been added at a level of 1g per 100g of soil (Lichtenstein, 1966).

2. Certain methylcarbamate compounds, some with insecticidal properties, have been shown to inhibit the enzymic hydrolysis of numerous phenyl-carbamate, acylanilide and acetamide type herbicides by soil micro-organisms, thus increasing their persistence and herbicidal lifetime in soil. The inhibition of CIPC metabolism in soil by the insecticide carbaryl has been reported, two to four fold increases in herbicidal persistence of CIPC being achieved (Kaufman et al., 1970). Detailed kinetic studies with purified enzymes from the soil bacterium Pseudomonas striata Chester, revealed that carbaryl competitively inhibits the carbamate hydrolysing enzyme (Kaufman et al., 1970). Inhibition of the microbial hydrolysis of CIPC by the non-insecticidal compound p-chlorophenyl methylcarbamate (PCMC) has also been demonstrated (Kaufman, 1977). PCMC has been commercially developed as an additive for CIPC, a mixture of the two compounds being manufactured by PPG Industries Inc. in the U.S.A. under the trade name "Furloe-124"

(Klingman et al., 1975). The addition of PCMC to CIPC formulations doubled the period of dodder control in field alfalfa with one CIPC application (Dawson, 1969; 1972). Methylcarbamates have been shown to increase the persistence of other herbicides containing carbamate linkages. Simultaneous application of PCMC and propanil has retarded the degradation of the herbicide in soil (Kaufman et al., (1971), again resulting from the inhibition of the acylamidase hydrolysing enzymes provided by soil micro-organisms that degrade propanil. Combination of carbaryl and propanil has resulted in similar effects (Bowling and Hudgins, 1966). PCMC has also inhibited the soil microbial metabolism of protham, CDAA, floumeturon and diuron (Kaufman, 1977) as well as that of barban (Wright and Forey, 1972) and isoproturon (Repioquet and Fournier, 1977).

Although the pathway of asulam degradation has not been elucidated as yet, hydrolytic cleavage of the carbamate linkage is a possible first step, similar to that of the carbamate based herbicides mentioned above. Thus an investigation into the effect of PCMC and carbaryl on asulam degradation in soil would seem justified.

The aims of this chapter were thus three-fold:-

- (1) To test the effect of a range of surfactant types on the adsorption, leaching and persistence of asulam in bracken soils. Emphasis was placed on an adsorption study similar to that carried out by Gaynor and Volk (1976) for picloram (see above).
- (2) To test the effect of methylcarbamates and other related compounds, on the degradation of asulam in soil.

- (3) To test the pre-emergence activity of asulam on bracken control, utilizing some, if any, promising manipulative treatments found in 1 and 2 above.

3.2 EXPERIMENTAL

3.2.1 Materials

Asulam was as previously described (2.2.1). Carbaryl (99.5% purity) was purchased from the National Physical Laboratory; tween 20, tween 85 and methyl carbamate from Koch Light Ltd.; sodium dodecylbenzene sulphonate, phenyl carbamate and p-chloroacetanilide from Phaltz and Bauer Inc. and cetyl trimethylammonium bromide from British Drug Houses Ltd. Sodium dodecyl sulphate was purchased as "Empicol" from Morchon Products Ltd. and recrystallised twice from ethanol (Schmidt, 1957) PCMC was kindly donated by PPG Industries Inc., Pittsburgh, U.S.A. All other reagents were purchased from either Hopkin and Williams Ltd. or British Drug Houses Ltd. and were of the purest grades available.

The soils used were A horizons of soils collected at three sites in West-Central Scotland. Two of the sites were bracken infested. The other site, although not covered by bracken at the time of collection, was from a soil series (Darleith) where infestation of bracken is common (Babiker, 1976). Brief description of the soils are as follows:-

Zoo Road: A deep organic-rich horizon of mull humus developed on a bank under bracken.

Carbeth: Organic-rich A horizon of a well drained brown forest soil of low base status developed on a hill slope under bracken.

Darleith: A horizon of a shallow brown forest soil of low base status developed on a gentle hill slope under grass.

Some relevant analytical data is presented in table 3.1.

Table 3.1 Analytical data of soils used in the study

Soil	pH	%OM	%sand	%silt	%clay	CEC
Zoo Road A	4.6	27	27	31	15	40
Carbeth A ^a	4.5	33	21	26	19	68
Darleith A ^b	5.5	21	17	26	35	40

%OM was by the Walkley-Black method (Walkley, 1942).

All other determinations were by standard soil analytical techniques (Jackson, 1962).

^a Data courtesy of Dr. M.B. Sommerville

^b Data courtesy of Dr. I.D. Pulford.

The soil samples were wet sieved through a 2mm sieve. Soil for the adsorption experiments was air-dried at 30°C in a forced draught oven before storage. Sieved soil destined for degradation studies was kept in sealed polythene bags at room temperature until use; moisture contents of the soils used were:- Carbeth 53.7% and Darleith 38.9%. These values were found not to vary over long term storage.

3.2.2 Methods

3.2.2.1 Adsorption experiments

Adsorption was determined by shaking 2g of air-dry soil with 10 ml of the appropriate asulam or blank solutions on an end-over-end shaker for 16h at $22 \pm 1^\circ\text{C}$ in a sealed 60 ml capacity bottle. The time period employed had previously been shown to be sufficient for maximum adsorption of asulam (Babiker and Duncan, 1977) and preliminary investigation substantiated this for the present conditions. The equilibrium pH was noted and the slurry filtered through Whatman No. 42 filter paper.

Analysis of the asulam remaining in solution was by a modification of the diazotisation and coupling (Bratton-Marshall reaction) procedure of Brocklesby and Muggleton (1973). 3 ml aliquots were taken and diluted to 10 ml prior to normal development of the Bratton-Marshall colour. The samples were then saturated with NaCl and extracted with 3 ml of n-Butanol prior to spectrophotometric determination. Preliminary work showed that this modification eliminated any interference from the surfactants when asulam is determined. The extraction of the Bratton-Marshall colour into n-Butanol has been used by other workers to concentrate samples for enhancing the sensitivity of the method for asulam persistence studies (Babiker and Duncan, 1977; Smith and Walker, 1977).

Since asulam adsorption is affected by pH (Babiker and Duncan, 1977) it was considered desirable to buffer the slurries to a constant pH in the present study. However, preliminary work showed that very strong buffering capacities of the surfactant solutions themselves

resulted in unacceptably high levels of buffer being necessary; levels which themselves were affecting asulam adsorption. Thus it was decided to omit the use of buffers but to note the equilibrium pH in all cases.

Adsorption was calculated by difference from that in solution before and after equilibration. All treatments were carried out in triplicate with the inclusion of appropriate blanks for aqueous and surfactant solutions. Data was subjected to analysis of variance, followed by LSD testing where appropriate.

Correlation of the equilibrium pH values noted and the degree of adsorption was carried out after the method of Snedecor and Cochran (1967).

Two adsorption experiments were carried out:

- (1) The influence of a range of surfactants on the adsorption of asulam was assessed on the soils. Asulam solutions (7.5 and 15 $\mu\text{g/ml}$) were prepared in water and 0.1, 1 and 10% w/v surfactant solutions prior to equilibration. The surfactants used were cetyl trimethylammonium bromide (cetab), a cationic surfactant; sodium dodecyl sulphate (SDS), an anionic surfactant; tween 20 - polyoxyethylene (20 moles) sorbitan monolaurate, a relatively hydrophilic non-ionic surfactant; and tween 85 - polyoxyethylene (20 moles) sorbitan trioleate, a more hydrophobic non-ionic surfactant. Tween 85 was not soluble enough at the 10% solution level and this concentration was omitted.
- (2) The influence of the anionic surfactant sodium dodecylbenzene sulphonate (SDBS) on asulam adsorption was assessed for the three soils. Asulam solutions of 7.5 and 15 $\mu\text{g/ml}$ were prepared in water and 0.1, 1 and 10% w/v surfactant solution prior to equilibration.

Due to the high viscosity of the 10% SDBS solutions, Whatman GF/A glass fibre filter paper was used at the filtration stage of this experiment.

In an experiment associated with the interpretation of the adsorption experimental results, the influence of pH and presence of surfactants on the partition coefficient of asulam in an n-octanol water system, as used by Babiker (1976), was carried out.

Asulam was prepared at 7.5 µg/ml in buffer (0.2M, prepared after Gomeri, 1955) at three pH levels (4-5.7). The hydrophilic-hydrophobic balance of asulam was assessed by shaking 10 ml of the solutions with 10 ml of n-octanol for 2h on an end-over-end shaker. After centrifuging at 3000 rpm for 10 min, the asulam remaining in the aqueous phase was determined by the method of Bratton and Marshall (1938).

Asulam behaviour in the presence and absence of a range of cetab and SDBS concentrations (0.1, 1 and 10% w/v) was assessed at two pH levels for each surfactant. The necessary level of buffer was added for each asulam/additive combination to achieve the pre-determined equivalent pH level.

Each treatment was carried out in duplicate.

3.2.2.2 Degradation studies

Asulam was added in 1 ml of water to 12.5g A.D.E. weight of soil in a 120 ml capacity amber glass screw topped bottle. One ml of water or the appropriate additive solution was then added prior to thorough mixing with a spatula. The recovery factor of asulam with and without additives was determined by taking samples immediately and

assaying the extracted asulam. Uniformity of distribution after mixing had also been checked in this way. A cotton wool plug was placed in the neck of each bottle and incubation carried out under non-leaching conditions at $22 \pm 1^\circ\text{C}$ for 8 days. The moisture content of each sample was maintained at the original level for each soil type (see 3.2.1) by adjusting to constant weight every second day.

Two bottles per treatment were employed with duplicate samples being drawn from each bottle.

The method of Babiker and Duncan (1977) was employed for asulam determination. Samples equivalent to 2g air-dry weight were shaken with 25 ml of acetate buffer at pH 5.6 (prepared after Gomeri, 1955) for 3h on an end-over-end shaker in 60 ml capacity screw topped bottles. After filtration through Whatman No. 42 filter paper, the Bratton-Marshall colour was developed in 10 ml aliquots before concentration by extraction into 3 ml n-butanol as before (3.2.2.1). The extracts were further checked by t.l.c. (Fishbein, 1967) for the possibility of interferences from other aromatic compounds, especially primary amines which will show up as positive under the determination conditions (Bratton and Marshall, 1939). No significant amount of interfering compounds were found in any extract. Recovery factors for the extraction procedure in Darleith and Carbeth soils were $92 \pm 1\%$ and $63 \pm 2\%$, respectively.

Three experiments were carried out:-

- (1) The degradation of asulam (1000 μg , equivalent to 80 $\mu\text{g/g}$ of air-dry soil) in Carbeth and Darleith A horizons was assessed in the presence and absence of a range of concentrations of surfactants (80, 800 and 8000 $\mu\text{g/g}$ of soil). The additives employed were cetab, SDS, tween 20 and tween 85.

- (2) The degradation of asulam (80 $\mu\text{g/g}$) in the Carbeth and Darleith soils was assessed in the presence and absence of a range of concentrations (80, 800 and 8000 $\mu\text{g/g}$) of the anionic surfactant SDBS.
- (3) The degradation of asulam (80 $\mu\text{g/g}$) in the Carbeth and Darleith A horizons were assessed in the presence and absence of two concentrations (X1 and X10 molar equivalents with asulam) of additive compounds which possess carbamate linkages. Both methylcarbamate and phenylcarbamate derivatives were included for comparison. The compounds employed were PCMC, phenylcarbamate, p-chloroacetanilide, methylcarbamate and carbaryl.

3.2.2.3 Influence of surfactants on the leaching of asulam down a soil plate

Leaching experiments were carried out according to a slightly modified version of the "soil thick-layer chromatography" method of Gerber et al. (1970). Preliminary experiments employing more conventional leaching columns (Harris, 1966; Smith and Bayer, 1967; Babiker and Duncan, 1975a) were found to be unsuitable due to lack of reproducibility, probably as a result of non-uniform soil packing and channelling between the soil and the walls of the glass columns containing the soil; the latter problem being accentuated in the presence of the surfactant solutions.

An aluminium plate, 30 x 5 cm, with 0.5 cm high walls along the two longer sides, was evenly covered with 80g of air-dried Zoo Road A horizon with a maximum particle size of 1 mm. Asulam was applied at a distance

of 3 cm from one end of the plate. A thin metallic frame 1 x 5 cm was inserted into the soil layer covering the cross-section where the herbicide was to be placed. The soil within the frame was sucked away by a vacuum pump and replaced by 2.5g A.D.E. of fresh soil into which 0.5 ml of the appropriate 1000 $\mu\text{g/ml}$ asulam solution had been added and evenly mixed. The frame was carefully removed and the plate placed in a Fisons Growth Cabinet (see 2.2.2.1) at $25 \pm 0.75^\circ\text{C}$ and 100% r.h. At the upper end of the plate a small piece of cotton cloth, pasted to the plate, dipped into a deionised water container and drew water for the development of the chromatogram. The plate was inclined at 3° to obtain slow movement of water down the soil layer. When 10 ml of water had been collected at the bottom, the plate was removed. The total quantity of water on the wet soil plate plus percolated water amounted to 70 ± 3 ml. The time taken for the whole leaching process was 175 ± 5 min.

Asulam position on the wet soil plate was immediately determined by carefully removing 2 cm sections along the plate into 60 ml capacity screw-topped bottles. Asulam content in the sections was determined by shaking with pH 5.6 buffer and developing the Bratton-Marshall colour as before (3.2.2.2).

Optical density at 540 nm was then plotted against position on the soil plate.

Asulam was applied to the fresh soil segment alone, or in the presence of cetab (0.1, 1 and 10%) or SDBS (0.1, 1 and 10%). Surfactant concentrations were 0.5, 5 and 50 mg/segment. The developing solution was water in each case. Two leachings per treatment were carried out.

3.2.2.4 Bracken field trial

This experiment was carried out at Carbeth, Stirlingshire (Grid Reference NS 527798) on a site known to support a vigorous stand of bracken. Spraying was by an I.C.I. Mark 3 Knapsack Sprayer at an output pressure of 30 p.s.i. Plot size was 5m x 5m with a 2m path between plots. A randomised block design with four replicates per treatment was employed. All bracken litter accumulations were removed from the plots prior to spraying.

The influence on bracken control of pre-emergence application of asulam alone and in combination with SDBS and cetab was assessed. Asulam was applied as "Asulox" (May and Baker Ltd. commercial formulation containing 40% w/v asulam as the sodium salt) at a rate of 16.8g/plot (6.7 kg/ha) in aqueous and 1% surfactant solutions. Some preliminary investigation had shown that higher surfactant levels started to become toxic to grass species. Appropriate controls were included. The total surfactant sprayed per plot was 10g (4 kg/ha). Spray volumes were 1l/plot (400l/ha).

Spraying took place on 10/5/79. Rain followed 3h after spraying, 4 mm falling in a 2h period.

Observations and assessment of control were carried out in the summer of the year of spraying and 1 year later. The mean number of fronds were counted in three 1 m² quadrats from the central 9 m² of each plot. The heights of ten fronds from the centre area were also measured.

3.3 RESULTS AND DISCUSSION

3.3.1 Adsorption studies

The interactions between the surfactant, herbicide and the various soil properties are very complex. To fully elucidate the reasons behind the degree of adsorption resulting under each particular condition would require a great deal of detailed experimental work far beyond the scope and reason of this chapter. Thus, only general trends will be commented on and some attempt made to rationalise these. The surfactants may specifically interact with asulam to alter its properties in solution and its adsorptive capacity. Alternatively, the surfactant may alter soil physical properties sufficiently to influence binding. It is likely that a complex combination of these factors will contribute to the overall effects observed.

Except for proportionately higher amounts adsorbed, asulam adsorption from surfactant solutions was similar at the 7.5 and 15 $\mu\text{g/ml}$ asulam concentrations, thus only values at 7.5 $\mu\text{g/ml}$ level are presented (tables 3.2 and 3.3).

Adsorption of asulam alone was generally lower in the Darleith soil than in the other two soils. This would be expected from their trends in organic matter content and pH - see 3.1 and table 3.1.

3.3.1.1 Cationic surfactant - cetab

Cetab had no effect on asulam adsorption at the lowest concentration tested (0.1%). A substantial increase in adsorption over the control occurred at the 1% surfactant level, representing 71-80% of the applied asulam being adsorbed. In this, the only case over all the

Table 3.2 Influence of various surfactants on the adsorption of asulam (7.5 µg/ml solutions) by the A horizons of three soils found under bracken

Treatment	Asulam adsorbed (µg/g)		
	Zoo Road A	Carbeth A	Darleith A
a)			
control (asulam alone)	24.2(1.3)	21.8(0.1)	10.8(1.3)
SDS 0.1%	** 20.8(1.3)	* 20.4(0.4)	11.4(0.7)
SDS 1%	*** 17.9(0.6)	*** 18.7(0.3)	** 7.8(0.2)
SDS 10%	*** 12.1(2.6)	*** 18.5(0.5)	*** 6.0(0.9)
b)			
control	22.6(0.6)	19.6(0.7)	9.6(1.9)
cetab 0.1%	24.0(0.8)	21.0(0.6)	13.2(0.3)
cetab 1%	** 28.5(3.4)	*** 27.5(0.3)	*** 28.2(0.3)
cetab 10%	21.1(2.5)	*** 13.0(0.1)	11.8(0.9)
c)			
control	22.1(0.2)	19.6(0.1)	10.4(1.3)
tween 20 0.1%	21.8(1.8)	19.6(1.0)	10.0(1.8)
tween 20 1%	*** 19.1(0.3)	** 23.4(2.0)	9.9(1.1)
tween 20 10%	22.2(0.4)	*** 14.7(0.2)	13.6(1.4)
d)			
control	23.0(0.4)	21.3(1.0)	10.9(0.7)
tween 85 0.1%	* 21.4(0.8)	18.8(0.8)	9.6(1.1)
tween 85 1%	*** 18.2(0.8)	* 17.4(2.1)	11.9(0.5)

Asulam values are means of 3 replicates. Figures in parenthesis are standard deviations (n-1 DF).

Treatment values significantly different from their asulam alone controls are indicated: * at the 5% level; ** at the 1% level; *** at the 0.1% level.

Table 3.3 Influence of SDBS on the adsorption of asulam (7.5 µg/ml solution) by the A horizons of three soils found under bracken

Treatment	Asulam adsorbed (µg/g)		
	Zoo Road A	Carbeth A	Darleith A
control (asulam alone)	21.0 (0.2)	19.5 (0.9)	10.6 (0.4)
SDBS 0.1%	18.2 (0.3)	*** 14.7 (0.9)	7.9 (0.1)
SDBS 1%	* 14.0 (3.7)	*** 11.5 (0.7)	7.9 (0.3)
SDBS 10%	** 13.2 (1.1)	*** 14.4 (1.0)	12.9 (5.6)

All details are as for table 3.2

treatments, the differences in adsorption due to pH and organic matter between the three soils were eliminated; the Darleith soil adsorbing asulam in equal amounts to the other two soils. At the highest concentration employed (10%), adsorption returned to control levels in the Zoo Road and Darleith soil. In the Carbeth soil a significant decrease in adsorption occurred (table 3.2).

The results are similar to those observed by Gaynor and Volk (1976) for the effect of the cetyl pyridinium chloride on the adsorption of the acidic herbicide picloram on a range of soils. As their soil pH's were relatively high (5.0-7.5), where picloram was charged, they interpreted these results as being that the anionic herbicide reacted with the positively charged detergent thus decreasing picloram solubility. The combined species was then readily adsorbed to the soil. At low surfactant concentrations, detergent molecules are adsorbed to cation exchange sites, neutralising the charge of the surfactant. At the 1% level, more surfactant charge is available to adsorb picloram. At the 10% surfactant concentrations, soils with higher organic matter adsorbed the surfactant chains to the hydrophobic surfaces which decreased the availability of the cationic charge. As the organic matter decreased, more cationic charge was available to adsorb anionic picloram (Gaynor and Volk, 1976). A similar argument for asulam could account for the fact that at the 1% level, adsorption was similar in the Darleith soil to that for the other two soils, and that when the cetab concentration was increased to 10%, a marked decrease in adsorption occurred, especially in the Carbeth soil. However, such an argument would require that much of the asulam was in the anionic form. When

one considers the low pH (4.5-5.5) of the soils involved, this would seem doubtful, however, in electrophoresis studies, Babiker and Duncan (1975a) have shown that asulam bears a negative charge down to a pH value between 3.0 and 3.9. On the other hand, one need also consider that acidic soils have a colloidal surface acidity one or two pH units lower than the soil solution pH because of hydrogen saturation (Mortland and Raman, 1968; Swoboda and Kunze, 1968). This would make the inflection point of the dissociation curve for asulam in soil systems less easier to define than in aqueous systems ($pK_a = 4.82$).

Smith and Bayer (1967), discussing a large increase in adsorption of diuron using various cationic surfactants, suggested that the positively charged detergent binds to the negative soil colloids, thus leaving layers of alkyl chains onto which the diuron is adsorbed by Van der Waals' forces, removing it from solution. At lower levels of surfactant, presumably there is not enough detergent to present sufficient adsorbant surfaces. Higher concentration of surfactant (10%) were not used here (Smith and Bayer, 1967). Asulam adsorption to organic matter occurs mainly through hydrophobic interactions (Carringer et al., 1975). This will be especially true for the pH values encountered in the present study (Babiker and Duncan, 1977). Thus, such a mechanism as proposed by Smith and Bayer (1967) could be appropriate for asulam-cetab-soil interactions.

Huggenberger et al. (1973), using non-ionic surfactants, observed a similar pattern of adsorptive response for lindane and diuron in soils. A slight increase in adsorption of the pesticides at low concentration ($< 0.05\%$) was considered due to dispersion of the

soil by the surfactants (Mustafa and Letey, 1968), increasing the number of adsorption sites, rather than an interaction between the surfactant and the pesticide molecule. The marked increase in adsorption at intermediate surfactant concentrations (0.1-0.2%) was a result of the surfactant concentrations being higher than the critical micelle concentration (c.m.c.) of the detergents in question. Above the c.m.c., in aqueous solution, surfactant molecules aggregate into micelles by arranging their lipophilic ends in the centre of the micelle (Becher, 1973). A certain proportion of the pesticide molecules were trapped in the centre of the micelles due to their lipophilicity. Significant adsorption of the micelles containing "trapped" pesticide, then took place (Valoras et al., 1969). At higher surfactant concentrations, proportionally less micelles out of the total in solution could be adsorbed, thus more micelles containing pesticides remained in aqueous solution, resulting in a decrease in adsorption (Huggenberger et al., 1973). The c.m.c. of cetab may occur near or just below the 1% level in solution, which may result in a high rate of adsorption of micellar, hydrophobic, asulam. Additionally, some slight negative charge on asulam may be attracted enough to the cetab molecule such that it is easily trapped on micelle formation.

One, or all, of the above mechanisms may be involved in the overall effect of cetab on asulam adsorption.

3.3.1.2 Anionic surfactants - SDS and SDBS

On the whole, the anionic surfactants decreased adsorption of asulam. Only in the Darleith soil at 0.1% was no effect from SDS observed (table 3.2). SDBS, at all levels, resulted in a decrease in

adsorption in the Carbeth soil. Variability was higher in the results of the other two soils and only in the Zoo Road soil, at the 1 and 10% detergent levels, was a significant decrease in adsorption noted (table 3.3). No significant increase in adsorption arose from any surfactant treatment in any soil.

Anionic surfactants are adsorbed to soils by mechanisms such as metal-ion bridging to clay and/or organic matter and interaction with positive clay faces and soil colloids with pH dependent charge (Wayman, 1963). Reductions in adsorption of picloram by anionic detergents on relatively high pH soils (pH 5.0-7.5) was considered to be due to competition for adsorption sites (Gaynor and Volk, 1976). For such a situation to exist for asulam, a preponderance of negatively charged species would seem necessary, the contributing amount of which was in question above (see 3.3.1.1). Coupled to this, the complexing power of negatively charged asulam to, e.g., calcium for metal-ion bridging to colloids is considered very low due to the steric hindrance of the charge on asulam which is localised at a nitrogen centred between a carbonyl and a sulphonyl group (Carringer et al., 1975). In general, repulsion of charged asulam by the predominantly negatively charged soil colloids is expected (Babiker and Duncan, 1975a; Babiker, 1976). Competition for organic matter sites, between hydrophobically bonded molecular asulam and anionic surfactants interacting through coulombic forces, may be occurring at low levels. As the surfactant concentration increased, any micelles formed will have their charges orientated outwards, thus tending to restrict any increase in adsorption by asulam onto hydrophobic surfactant tails. The micelles are still bound to soil by their negative charges.

Little micellar "trapping" would appear to be occurring, perhaps due to a slight negative charge on the asulam repulsing the anionic surfactant prior to micelle formation. Inevitably, at higher surfactant concentrations, some increase in surfactant adsorption will occur resulting in enhanced asulam adsorption. Such behaviour was less apparent than with cetab which may be due to a weaker interaction between asulam and the hydrophobic surfaces, compared to that with cetab. Smith and Bayer (1967) reported differences in the hydrophobic adsorption of diuron to cation detergents of different alkyl chain lengths. Differences in the extent of surfactant adsorption will also be reflected.

3.3.1.3 Non-ionic surfactants - Tween 20 and Tween 85

Adsorption of molecular and micellar non-ionic surfactant to the soil would be expected (Valoras et al., 1969). Trends in behaviour similar to those observed by Huggenberger et al. (1973) for diuron and lindane (see 3.3.1.1), were only observed for tween 20 in the Carbeth soil, i.e., enhanced adsorption at the 1% level followed by a decrease at 10%. As this soil had the lowest equilibrium pH values (see table 3.4), this may have been the only situation where asulam was sufficiently lipophilic to become trapped in the surfactant micelles. The exact c.m.c. and differences between the two surfactants would need to be ascertained here for firm conclusions to be drawn. In the other cases where significant differences in adsorption occurred, these were reductions. This is opposite to the effects found by Gaynor and Volk (1976) for non-ionic surfactants on picloram adsorption. No effect by these surfactants on the lower organic matter, higher pH, Darleith soil, was observed (table 3.2).

3.3.1.4 Related pH and asulam solubility studies

As mentioned earlier (3.2.2.1), the pH of the equilibrations were not buffered. It could thus be argued that changes in the solution pH values by the individual surfactants may have been a significant factor resulting in the changes in asulam adsorption behaviour observed. The equilibrium pH values noted throughout all the experiments are given in table 3.4. Correlation analysis over all the data revealed that adsorption was not significantly related with the resultant pH values on each soil type. The cetab and tween solutions resulted in very little changes in pH over the non-surfactant mixtures; certainly none that would result in the marked adsorption changes induced by, e.g., cetab (1%). The anionic surfactants employed, especially SDBS, tended to increase the pH values, which could be expected to reduce the adsorption (Babiker and Duncan, 1977), as observed here (3.3.1.2).

Changes in the hydrophilic-lipophilic balance of asulam occur with changing pH. This was illustrated in the n-octanol/water partition coefficients for asulam in the absence of surfactants (table 3.5). The anionic species will favour the aqueous phase due to hydrogen bonding with water (Kipling, 1965). At pH 4.0 (below the pKa), asulam would be expected to be mainly as the molecular species. A high partition coefficient was observed here. The presence of cetab at this pH had no effect at the lowest detergent level tested (0.1%), whereas, with the other two levels (1 and 10%) solubility was increasingly reduced. The surfactant molecules would be expected to migrate to the interphase, probably carrying hydrophobically bound asulam in an increasing amount as the detergent level rose. At pH 5.0, where more charged asulam would be

Table 3.4 Equilibrium pH values noted in the surfactant-asulam
adsorption experiments

Treatment	Equilibrium pH		
	Zoo Road A	Carbeth A	Darleith A
control (no surfactant)	4.6	4.4	5.4
SDS 0.1%	4.6	4.4	5.0
SDS 1%	4.6	5.3	5.4
SDS 10%	4.8	5.3	5.4
SDBS 0.1%	5.4	5.0	5.6
SDBS 1%	5.6	5.4	5.7
SDBS 10%	6.1	5.7	6.1
cetab 0.1%	4.6	4.2	5.4
cetab 1%	4.6	4.5	5.1
cetab 10%	4.2	4.2	5.0
tween 20 0.1%	4.6	4.4	5.4
tween 20 1%	4.6	4.4	5.3
tween 20 10%	4.3	4.4	5.2
tween 85 0.1%	4.6	4.4	5.4
tween 85 1%	4.3	4.3	5.4

Correlation of all adsorption data with equilibrium pH values:

Zoo Road $r = -0.57$; Carbeth $r = -0.40$; Darleith $r = -0.29$.

Table 3.5 Influence of pH and presence of surfactants on the partition coefficient of asulam in a n-octanol-water system

Additive	pH	Partition coefficient <u>n</u> -octanol/water			
		additive concentration %			
		0	0.1	1	10
cetab	4.0 ^a	1.08(.08)	1.06(.04)	1.36(.21)	1.52(.11)
cetab	5.0 ^a	0.39(0)	0.40(.02)	0.68(.02)	0.94(.04)
SDBS	5.0 ^a	0.39(0)	0.23(.02)	0.36(.04)	0.50(0)
SDBS	5.7 ^b	0.14(0)	0.02(0)	0.01(.02)	0.16(0)

^a buffer used sodium acetate, acetic acid 0.2M (Gomeri, 1955).

^b buffer used Na_2HPO_4 , NaH_2PO_4 0.2M (Gomeri, 1955).

Figures in parentheses are standard deviations (n-1 DF).

The LSD after Analysis of Variance was 0.11.

available to precipitate out with cetab, no decrease in solubility was observed. Unfortunately, cetab could not be buffered, in realistic amounts, at higher pH values to follow this further. At pH 5.7, where asulam was predominately charged, low partitioning was observed. SDBS increased solubility of asulam at the two lowest levels, whereas, at the highest level tested (10%), this effect was nullified. The reduced partitioning may be due to orientation of the surfactant at the inter-phase with the negative head repelling asulam ions. The trend towards decreasing solubility at the higher surfactant concentration, may be due to micellar "trapping". At pH 5.0 the partitioning trends for SDBS treatments were similar, with an increase in solubility at the 0.1% level and a decrease at the top level; solubility overall was decreased compared to pH 5.7. On contrasting the effects of the two surfactants at a constant pH 5.0, the differences in the activities of the two detergents are noteworthy. They show, at least, that the properties of the surfactant itself will probably be a contributing factor to the solubility and adsorptive behaviour of asulam in soil solutions, as well as any effect they may have on the equilibrium pH.

Throughout their experiments with picloram, Gaynor and Volk (1976) made no attempt to buffer the equilibrium pH, neither did they make any observations on the effect of their surfactant solutions on this. They based their discussion solely on the 1:1, soil to water, pH values determined beforehand. This does tend to call into question many of their results and conclusions.

3.3.2 Degradation studies

Degradation was greater in the Carbeth soil than in the Darleith soil (tables 3.6-3.8), as would be expected from the higher organic matter and moisture content of the former (see 3.1 and table 3.1).

3.3.2.1 Surfactant additives

At all three levels tested, SDS increased the degradation rate of asulam in the Carbeth soil. In the Darleith soil, the two highest levels also enhanced the degradation rate while the lowest level (80 µg/g) had no effect (table 3.6). In view of the protein denaturing and bacteriocidal properties of SDS (Putman, 1948), it had been considered that this compound was potentially promising for reducing the rate of asulam disappearance. It appears, however, that SDS is too rapidly degraded itself (Swisher, 1970), resulting in greatly enhanced microbial activity which, in turn, leaves asulam open to increased attack. Mycelia were visible in the incubation bottles containing the highest SDS concentration (8000 µg/g) in both soils, indicating a large increase in microbial population.

Because of the adsorption reducing properties of SDS, it was decided at this stage to look for another, less biodegradable anionic surfactant, which may possess those properties without adversely influencing the degradation rate of asulam. SDBS is less biodegradable than SDS, but not so much so that an environmental hazard would result (Swisher, 1970). Hence the use of SDBS here and in the other investigations of this chapter. At all levels tested, this surfactant

Table 3.6 Influence of various surfactants on the degradation of asulam (80 µg/g A.D.E.) in the A horizons of two soils found under bracken

Treatment	Asulam remaining (µg/g A.D.E.)	
	Carbeth A	Darleith A
control asulam alone)	12.3 (0.2)	27.4 (0.4)
SDS (80)	* 10.2 (1.0)	26.9 (0.8)
SDS (800)	*** 7.8 (0.7)	** 24.5 (0.5)
SDS (8000)	*** 7.8 (1.2)	*** 13.8 (0.9)
cetab (80)	13.3 (2.3)	28.7 (1.6)
cetab (800)	11.8 (1.1)	29.0 (1.2)
cetab (8000)	** 9.0 (1.2)	*** 23.0 (0.4)
tween 20 (80)	10.9 (0.3)	25.7 (0.7)
tween 20 (800)	11.5 (0.9)	* 25.0 (0.9)
tween 20 (8000)	11.5 (0.2)	*** 21.3 (0.6)
tween 85 (80)	* 10.1 (0.7)	*** 23.7 (0.8)
tween 85 (800)	10.4 (0.3)	* 24.9 (0.5)

Additive treatments are in µg/g of soil, representing 0.1, 1 and 10% stock solutions in which the asulam was added to the soils. Asulam values are means of 4 samples taken from duplicate incubations. Incubation period was 8 days at $22 \pm 1^\circ\text{C}$. Figures in parentheses (asulam values) are standard deviations (n-1 DF). Values significantly different from the control are indicated; * at the 5% level; ** at the 1% level; *** at the 0.1% level.

produced no change in the rate of disappearance of asulam in either of the two soils (table 3.7).

Cetab is also well known for its bacteriocidal and germicidal properties (Swisher, 1970). This detergent did not influence the rate of disappearance of asulam, except at the 8000 $\mu\text{g/g}$ level where a significant increase in degradation rate occurred in both soils (table 3.6). Non-significant decreases in asulam degradation at low cetab concentrations could have been due to more asulam adsorption taking place, thus providing protection from degradation. The contribution which such an effect has on the overall process of pesticide degradation in soils is not clear and considered doubtful (Walker, 1977).

At all three levels tested, tween 20 had no effect on asulam degradation in the Carbeth soil. In the Darleith case, both the two highest levels (800 and 8000 $\mu\text{g/g}$) enhanced disappearance (table 3.6).

Tween 85 (80 $\mu\text{g/g}$) significantly increased the rate of breakdown of asulam in the Carbeth soil. Both levels tested (80 and 800 $\mu\text{g/g}$) enhanced disappearance in the other soil (table 3.6).

On the whole, the surfactants tested increased the degradation rate of asulam. With the exception of SDS, this effect was greater in the Darleith soil than in the Carbeth. This may be due to the additional source of carbon presented by the surfactants enhancing microbial growth, and so asulam degradation, to a more significant degree in the lower organic matter Darleith soil.

Table 3.7 Influence of SDBS on the degradation of asulam
(80 $\mu\text{g/g}$ A.D.E.) in the A horizons of two soils found under
bracken

Treatment	Asulam remaining ($\mu\text{g/g}$ A.D.E.)	
	Carbeth A	Darleith A
control (asulam alone)	13.9 (0.9)	27.1 (0.7)
SDBS (80)	14.4 (0.8)	27.7 (0.8)
SDBS (800)	14.4 (0.3)	26.6 (1.6)
SDBS (8000)	13.8 (1.0)	25.7 (1.2)

Details are as for table 3.6. No significant differences were observed.

3.3.2.2 Carbamate additives

None of the carbamate derivatives tested had any significant effect on the rate of degradation of asulam in the two soils (table 3.8).

It could be concluded that conversion to the amide is not a major degradative pathway for asulam in soil. This was suspected, since, when checking the colorimetric analytical procedure, no sulphanilamide was found to be present after 8 day incubations (see 3.2.2). Steric hindrance or electron withdrawal by the adjacent sulphonyl group may effectively inhibit the action of the microbial amidases on the carbamate linkage. Oxidation of the aromatic amine group, which the Bratton-Marshall reaction determines, may thus be a faster, and hence more important, degradation reaction than the amidase pathway. This would make any activity of the carbamate additives academic.

There are other factors which have to be considered before a combination of chemicals, such as those attempted here, could be applied in practice. Important considerations would be; a) physical compatibility of the chemicals involved; b) additive phytotoxicity; c) additive degradation rate in soils; d) additive movement in soils compared to the pesticide molecule; e) additive toxicology; f) the relative rates of herbicide and additive uptake and translocation in both target and crop plants; and g) the economics of the combination.

Table 3.8 Influence of various carbamate additives on the degradation of asulam (80 µg/g A.D.E.) in the A horizons of two soils found under bracken

Treatment	Asulam remaining (µg/g A.D.E.)	
	Carbeth A	Darleith A
control (asulam alone)	16.1 (0.4)	34.0 (0.2)
PCMC (65)	15.8 (0.3)	35.0 (0.1)
PCMC (645)	15.2 (1.3)	31.0 (4.1)
phenylcarbamate (48)	16.3 (0.4)	34.7 (0.5)
phenylcarbamate (476)	16.2 (0.6)	34.8 (0.7)
p-chloroacetanilide (59)	17.1 (2.6)	34.5 (0.5)
p-chloroacetanilide (590)	15.8 (0.3)	35.4 (0.8)
methylcarbamate (26)	16.8 (1.4)	38.9 (6.7)
methylcarbamate (261)	16.6 (1.2)	35.5 (1.5)
carbaryl (70)	17.1 (0.3)	40.0 (0.3)
carbaryl (699)	17.2 (2.3)	36.2 (2.6)

Additive treatments are in µg/g of soil, representing X1 and X10 molar equivalents with asulam. Asulam values are means of four samples taken from duplicate incubations. Incubation period was 8 days at $22 \pm 1^\circ\text{C}$. Figures in parentheses (asulam values) are standard deviations (n-1 DF). No significant differences were observed.

3.3.3 Leaching experiments

Figure 3.1a shows that asulam is mobile in the Zoo Road soil. The position of maximum asulam concentration was 14 to 16 cm down the soil plate and 7.0 μg had eluted with the solvent front (table 3.9). This result supports the work of Babiker and Duncan (1975a) who showed asulam mobility in soil and stated that in freely drained soils, asulam could possibly result in environmental problems. In the present study, conducted on an acid and highly organic soil, adsorption would be expected to be at or near a maximum and yet the chemical is still mobile. However, the capacity of the soil to degrade asulam may reduce the quantity and depth to which the chemical will leach under practical field conditions (Helling, 1970; Hamaker, 1974; Babiker and Duncan, 1977).

As a result of their contrasting influence on asulam adsorption, SDBS and cetab were used in an attempt to gain some degree of alteration in asulam movement. There was virtually no difference in the elution pattern with the 0.1 and 1% surfactant levels over the control; thus only the 10% treatments are illustrated (figure 3.1b-c). As would be predicted from its adsorptive behaviour (3.3.1.2), SDBS (10%) tended to enhance the rate of leaching, with a concentration maximum 18-20 cm from the top of the plate and 11.2 μg in the run-off. Cetab (10%) slowed the rate of leaching and lowered the subsequent drainage water concentration (10-12 cm and 4.2 μg , respectively) - see table 3.9.

The fact that the surfactant treatments only had an effect at the highest concentrations tested tends to illustrate where problems may lie in the use of detergents in controlling pesticide movement in soil.

Table 3.9 Influence of surfactants on the leaching characteristics of asulam on a soil plate (Zoo Road A horizon)

Treatment	Position of maximum asulam concentration ^a	Total asulam in drainage water ^b
control (asulam alone)	14-16	7.0 (0.4)
SDBS 0.1%	14-16	7.1 (0.6)
SDBS 1%	14-16	6.9 (0.4)
SDBS 10%	18-20	11.2 (0.9)
cetab 0.1%	14-16	7.1 (0.6)
cetab 1%	14-16	7.0 (0.8)
cetab 10%	10-12	4.2 (0.2)
LSD (5%)		1.4

^a position in cm from top end of soil plate

^b μg in 10 ml of run-off water from duplicate runs, values in parentheses are standard deviations (n-1 DF).

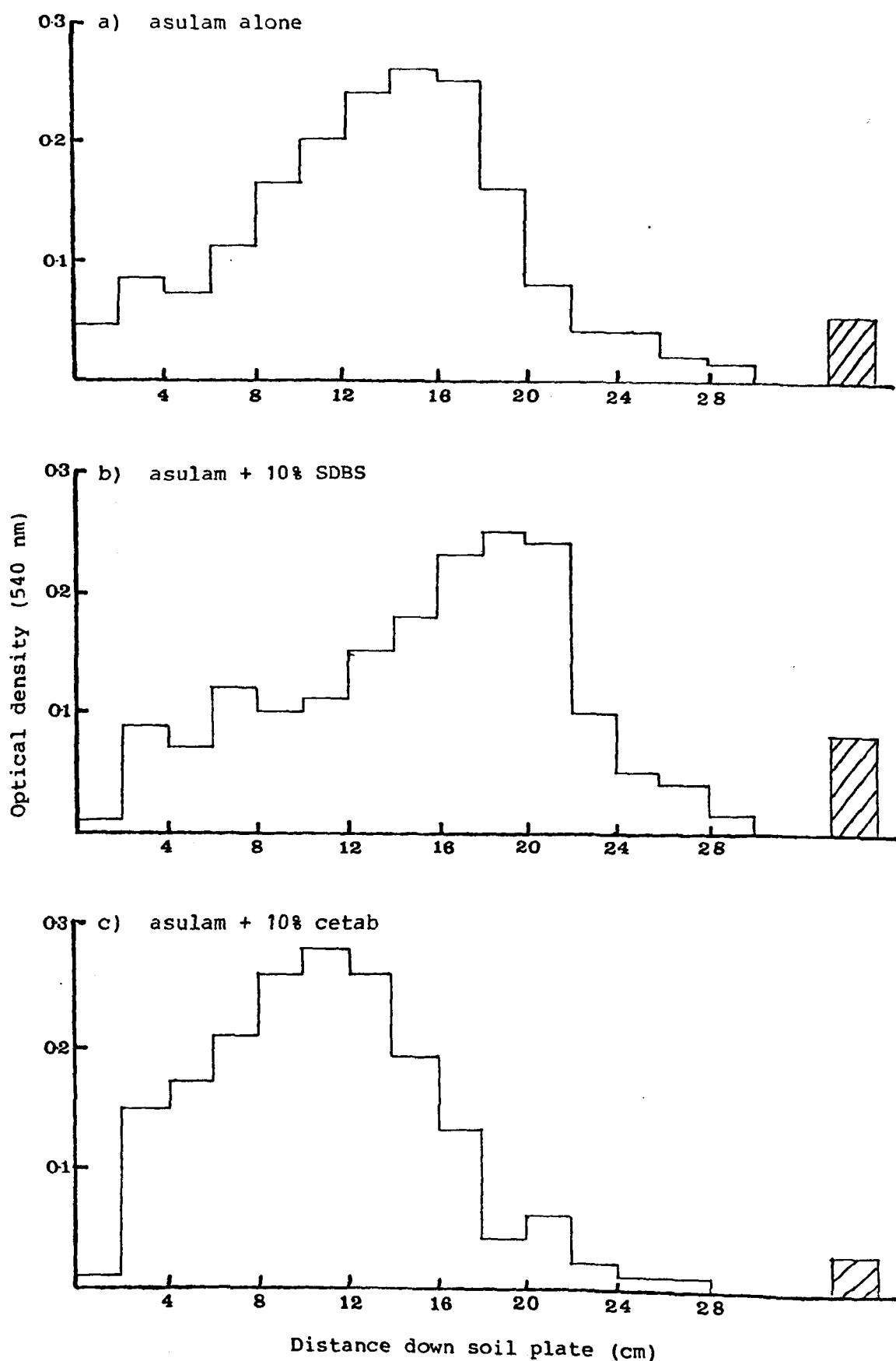


Figure 3.1 Influence of surfactants on movement of asulam down a soil (Zoo Road) plate. ▨ indicates quantity of asulam leached off plate.

The dilution of the surfactants by soil and precipitation water will result in low surfactant concentrations under practical soil conditions (Huggenberger et al., 1973). The adsorption experiments carried out here, and universally, are quite artificial in that the level of soil is low and the contact of that soil with a high volume of solution is very much greater than in real situations. They are, however, useful as guides to pesticide behaviour in soils if carried out in a uniform and controlled manner. It should be remembered that the effect of adsorption, etc., on herbicidal placement and availability should be viewed as dynamic rather than in a static, isolated way (Osgerby, 1973). The behaviour and fate of a herbicide in soil is determined by the interactions of important factors with the total environment rather than with a single aspect or factor (Ogle and Warren, 1954; Babiker, 1976). The number of adsorption sites in soils are thousands of times greater than the number of pesticide molecules applied (Riley and Morrod, 1976). Adsorption and degradation of the surfactant molecules themselves need also be considered (Riley and Morrod, 1976), the soil being basically a chromatography column separating herbicide from additives (Osgerby, 1973). On the whole, uneconomically large quantities of formulation additives would probably be required to control movement of pesticides in soil.

It is noticeable from the earlier review (3.1), that the only surfactant-pesticide-soil treatment that had proved successful under field conditions appeared to be the use of tween 20 on enhancing benomyl activity against Verticillium in potato (Biehn, 1970) and cotton (Rawlings and Booth, 1968). It may be that some highly specific

and significant interaction may occur between these two chemicals in the formulation, before application, which allows them to remain together in soil. Regulation of pesticide movement in soil with surfactants may only be practically possible if such a specific interaction occurred, the soil properties of the surfactant-pesticide complex becoming all important. Ability to survive dilution and degradation and for the pesticidal component to remain available for plant uptake, would be necessary. It may be that only in special situations that such combinations could be of value; perhaps soils of low organic matter or pH extremes, or in low rainfall furrow irrigation situations where more control over soil water content and movement can be gained.

3.3.4 Bracken field trial

No significant effects on bracken control by asulam, either alone or in combination with surfactant solutions, were observed in the season of spraying or one year afterwards (table 3.10). Observations in the months following spraying showed neither delayed emergence nor abnormal growth habit.

The asulam treatments resulted in almost complete kill of the grass sward in the season of spraying, however, a significant recovery had taken place by the following summer. It should be noted that as accumulation of litter had been removed from the plots just before spraying, this may have enhanced the susceptibility of the grasses to the asulam treatments. The surfactant treatments alone had no effect on the grass sward, neither did they enhance asulam toxicity. Any high degree of grass kill would be unacceptable, unless a full

Table 3.10 Influence of pre-emergence applications of asulam (6.7 kg/ha) and asulam-surfactant combinations on the control of bracken

Treatment	MFD(0) ^a	MFD(1) ^a	MFH(0) ^b	MFH(1) ^b
control	65(11.6)	72(5.7)	112(8.7)	104(6.1)
asulam	60(6.6)	69(11.2)	100(6.3)	114(5.0)
asulam + SDBS	66(9.2)	68(6.0)	97(10.0)	98(9.1)
asulam + cetab	61(6.3)	67(14.2)	115(11.1)	117(12.3)
SDBS	70(8.3)	69(16.7)	111(4.3)	102(12.2)
cetab	61(10.9)	65(7.5)	114(8.0)	110(7.5)

^a Mean frond density in season of spraying (0) and one year following application (1)

^b Mean frond height in season of spraying (0) and one year following application (1)

Figures in parentheses are standard deviations (n-1 DF).

Surfactant levels were 4 kg/ha.

No significant differences observed.

post-spraying program of reseeding by direct drilling or surface sowing was planned, all of which would add to the cost of reclamation.

Asulam would thus seem of little promise as a pre-emergence treatment for bracken. Further experimentation would be necessary with a number of concentrations on a wide range of soil types. The herbicide would have to perform adequately over a wide range of soil types. The soil used here (Carbeth), although fairly typical of those found under bracken in this area (Jarvis, 1974), was perhaps one of the most difficult targets that the chemical might have to face, in view of its soil behaviour (see 3.1).

As demonstrated here, the use of surfactants and other chemical additives to achieve more flexibility over the behaviour of asulam in soil would seem limited, at least in this acid-organic soil. They may have more value under other, less severe, conditions.

CHAPTER 4

AMMONIUM THIOCYANATE AND OTHER COMPOUNDS AS ADDITIVES IN
BRACKEN CONTROL CHEMICAL FORMULATIONS4.1 INTRODUCTION

The problems associated with the use of foliar-applied herbicides for bracken control and the desire to improve their effectiveness by the use of additives was discussed in chapter 1. The use of additives to improve the foliar uptake of herbicides in general has been reviewed by Kanellopoulos (1974) and Cook (1979), while discussion with particular relevance to the bracken situation was made by Babiker (1976), Cook (1979) and Williams (1980). The possible role of additives to perform other functions such as improvement of spray retention and translocation and reducing detoxification was introduced in chapter 1. This chapter deals with the role of the free radical scavenger ammonium thiocyanate (NH_4SCN) in decreasing herbicide detoxification and the development of a rapid assessment method to aid here and with the assessment of other possible scavenger additives.

The relevance of aminotriazole in bracken control has been discussed in chapter 1. In general weed control, the formulation of aminotriazole with NH_4SCN has been shown to result in a synergistic response (Crafts, 1961). Improved bracken control using this activated aminotriazole rather than aminotriazole alone, has been demonstrated (Anon, 1963, 1964; Varlet et al., 1964). Cook (1979), in this laboratory, also showed improved control using activated aminotriazole.

In these latter trials it was observed that those treated with the commercial formulation (Weedazol-TL, purchased from A.H. Marks and Co. Ltd. and which contains a 1:1 molar ratio of aminotriazole: NH_4SCN) had produced a slight necrotic scorch of the bracken fronds, while all aminotriazole alone treated plots were heavily scorched. From the results it appeared that reduced scorching was accompanied by increased control the following season and that NH_4SCN was the governing factor in this process. It was considered unlikely that rapid uptake had caused this scorching since 3 days after spraying there was still a considerable quantity of aminotriazole on the frond surface and traces after 4 to 5 days. This was followed after 6 days by sufficient rain to remove any residue if present. It was taken that in this instance at least, translocation, not penetration was the limiting factor and that enhancement by NH_4SCN had taken place (Cook, 1979).

Improvement of both uptake and translocation of aminotriazole by the addition of NH_4SCN has been implicated in plants in general, although there has been conflicting evidence put forward. Babiker and Duncan (1975) demonstrated increased uptake into bracken while Donnalley and Ries (1964) observed increased translocation but not uptake in couch grass (Agropyron repens L. Beauv). They proposed that this resulted from reduced damage at the absorption site, thereby allowing prolonged uptake and translocation. In contrast to this, however, Forde (1966) showed a decrease in translocation of ^{14}C label in couch grass treated with ^{14}C -aminotriazole applied in the presence of NH_4SCN . The formation of aminotriazole conjugates with endogenous amino acids and sugars is considered a major detoxification mechanism in higher plants (Ashton and

Crafts, 1975). Carter (1975) has shown that NH_4SCN inhibits the formation of the major metabolite 3-ATAL (3-[3-amino-1,2,4-triazole-1-yl]-2-aminopropionic acid) - see figure 4.1. This compound is a conjugate

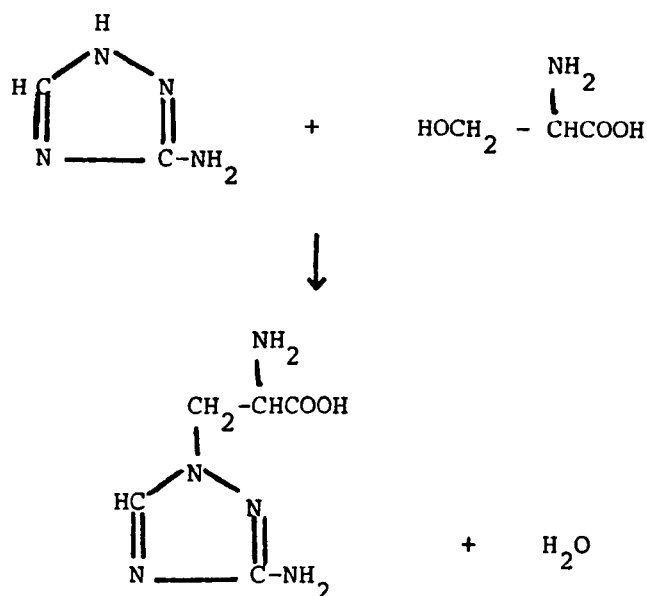


Figure 4.1 Aminotriazole conjugation with serine to form 3-ATAL.

of aminotriazole and serine and appears to be both less toxic and less mobile than aminotriazole (Racusen, 1958 ; Massini, 1963). The increased mobility of aminotriazole in the presence of NH_4SCN (Donnalley and Ries, 1964) is probably a secondary result of reduced 3-ATAL formation (Carter, 1975). The formation of aminotriazole free radicals by riboflavin, or other endogenous free radical generating systems, is thought to be a first stage in the aminotriazole detoxification pathway (Castelfranco and Brown, 1963; Brown and Carter, 1968; Carter, 1975), the free radical being available to react with serine and form 3-ATAL

(Castelfranco and Brown, 1963). SCN is known to be a free radical scavenger, working via a charge transfer mechanism (Adams et al., 1972; Bellus, 1978). Cook and Duncan (1979), in this laboratory, demonstrated that in two in-vitro free radical generating systems, SCN would inhibit the oxidation of aminotriazole by being preferentially oxidised and so inhibiting the formation of the aminotriazole free radical intermediate. The rapid formation of conjugate, via the aminotriazole free radical, is thought to be responsible for the scorch of bracken fronds induced by aminotriazole (Cook, 1979) and thus less overall movement of aminotriazole to the rhizome system. Field observations have indicated that the formation of aminotriazole scorch on the fronds after spraying is inversely related to the subsequent degree of control (Babiker, 1976; Cook, 1979). Thus, the possibility opened up^{OF} a whole new range of additives that could effectively enhance the efficiency of translocation by inhibiting free radical reactions near the site of absorption.

SCN has been found by chemists to exhibit many of the properties of the halides and so is often classified by them as a pseudohalide (Hughes, 1975). Thus, in addition to SCN, the halide anions were investigated as potential additives for aminotriazole formulations (Cook and Duncan, 1979). This study revealed that iodide(I) was also effective in two in-vitro free radical generating systems in terms of inhibiting aminotriazole oxidation. Of the other halides tested, bromide (Br) showed this ability in only one system while Chloride (Cl) and fluoride (F) gave little or no aminotriazole protection. The related anions cyanide (CN) and ferrocyanide ($\text{Fe}^{\text{II}}(\text{CN})_6$) were also effective in the one generating system tested (Cook and Duncan, 1979).

Of the additives which were successful in-vitro with aminotriazole, the obvious one to try in-vivo was I, while in comparison to CN it was obviously much safer. A field trial was set up to investigate the effects of I on aminotriazole activity on bracken. In both the presence and absence of aminotriazole, NaI caused extremely heavy black scorching. In the presence of NaI, aminotriazole effects were completely nullified the following year - see Cook et al., 1982. The heavy scorching brought about by NaI probably inhibited translocation to a large extent. These results were disappointing and stressed a need for a rapid bioassay which would determine the effects of possible additives for aminotriazole formulations, in terms of decreasing aminotriazole scorch and assessing their own phototoxicity, prior to field trials.

The object of the work of this chapter was to continue the work by, initially, developing such a bioassay. It was originally intended to use dwarf French bean as the test plant since, under field conditions, Van der Zweep (1965) had demonstrated similar responses to those found in bracken, however, these could not be reproduced in growth chamber grown plants. Further preliminary investigation revealed no suitable laboratory grown test species. As it was necessary to use field grown material of some sort, it was decided to try and use bracken since it was the ultimate target plant. It was found that by using segments collected from mature fronds, aminotriazole scorch could be reproduced.

A range of studies were carried out utilising the bioassay:-

(1) Further investigation into the scorch reducing properties of NH_4SCN in combination with aminotriazole. Two aspects were covered; a) a study using various relative concentrations of aminotriazole to NH_4SCN to determine if the commercial formulation ratio of 1:1 was optimum for bracken on one particular site; b) the influence of site environment on the degree of scorching induced by aminotriazole and its reduction by NH_4SCN .

(2) A range of compounds were tested for scorch reducing properties. These included a) NH_4 salts, to assess any effect of the NH_4 ion; b) SCN salts; c) compounds related to SCN 's such as CN , $\text{Fe}^{\text{II}}(\text{CN})_6$ and ferricyanide ($\text{Fe}^{\text{III}}(\text{CN})_6$) salts; d) various halides; e) various phenolic compounds, since many are known to inhibit free radical reactions (Pryor, 1976); f) organic sulphur compounds which have been shown to influence conjugation of aminotriazole (Carter, 1975).

The bioassay was also extended to other systems rather than the aminotriazole/ NH_4SCN on bracken leaflets:-

(3) Asulam has also been shown to be degraded by two in-vitro free radical generating systems, the oxidation being inhibited by SCN and I^- (Cook, 1979). This compound was thus incorporated into the bracken bioassay to determine if its control of bracken may be improved by the use of NH_4SCH in formulations. Holroyd and Thornton (1978) state that no scorch, or very little, by asulam is observed in the season of spraying. However, the possibility exists that the detoxification of asulam by conjugation may be manifested differently, or to a different degree, than that of aminotriazole on bracken.

(4) Docks (Rumex spp.) are a major weed of grassland. A recent survey in England and Wales showed serious infestation in 4% of swards by R. obtusifolius and R. crispus. The problem was mainly on intensive dairy farms and is associated with high nitrogen and phosphate applications (Peel and Hopkins, 1981). Good control of many broad-leaved weeds, including docks, has been achieved with aminotriazole (Joice and Norris, 1962). Aminotriazole is recommended in the UK for dock and couch grass control in ground to be used for swede or kale cropping (Fryer and Makepeace, 1978). Hull (1962) observed improved control of R. crispus using activated aminotriazole rather than aminotriazole alone.

The volunteer potato or groundkeeper (Solanum tuberosum L.) is becoming an important weed of British agriculture, particularly in areas of intensive potato production (Lutman and Richardson, 1978). Some control of groundkeepers using aminotriazole has been achieved (Lutman and Richardson, 1978).

The scorching ability of aminotriazole/ NH_4SCN formulations on these two weeds was bioassayed. In both cases, like bracken, translocation to the underground storage tissue would be necessary for their control.

On the basis of some of the bioassay results, bracken control field trials were carried out using various aminotriazole/additive and asulam/additive formulations.

4.2. EXPERIMENTAL

4.2.1 Herbicide scorch bioassays

Bracken pinnules from the second lowermost pair of pinnae were collected from fronds which were fully unfurled. These were transferred to self-sealing polythene bags, taken to the laboratory and used immediately. Five leaflets (first segmentations of pinnules) were floated adaxial surface uppermost in petri dishes (9 cm diameter) containing the herbicide and one of the range of additives under test in a total volume of 22 ml. Two replicate dishes per treatment were employed although each experiment was repeated at least twice to ensure that the trends obtained were constant. These were then transferred to a Fisons Growth Cabinet (Model 600 G3/THTL) at $21 \pm 0.75^{\circ}\text{C}$ and 16h day length with 21 klux light intensity supplied by warm white fluorescent tubes. After 72h, the degree of scorching of the leaflets was visually assessed on an arbitrary 0 to 5 or 0 to 10 scale, 0 = no scorching, 5 or 10 = complete scorching. More quantitative methods of assessment were considered but found to have no obvious advantages.

Dock (Rumex obtusifolius) leaves were collected from weed populations found locally in a neglected grassland area. Leaves 15-25cm in length were taken from plants which were still to flower. Selection of leaves suitably low in disease and insect damage was made. The chosen leaves were transferred immediately to the laboratory in sealed polythene bags and used immediately. Discs 17 mm in diameter were cut with a cork borer (No. 12), care being taken to avoid damaged

areas. Up to six discs per leaf were obtained. The discs were then assayed as for bracken leaflets. Assessment was made 120h after treatment.

Potato leaf discs were prepared and assayed as for docks. The leaves were taken from the upper regions of plants which had just flowered. The potatoes (c.v. Record) were grown as part of another experiment grown under commercial husbandry conditions at the University Field Station, Garscube, Glasgow.

4.2.1.1 Influence of ammonium thiocyanate on scorching of bracken leaflets by aminotriazole

Three sets of experiments were designed: a) leaflets were floated on aminotriazole solutions (3,6 and 12 mg/dish) in the presence and absence of equimolar concentrations of NH_4SCN ; b) leaflets were floated on aminotriazole solutions (6 mg/dish) in the presence of a range of NH_4SCN concentrations (2.7, 5.4, 10.9 and 21.7 mg/dish - molar ratios of 0.5:1 to 4:1 NH_4SCN :aminotriazole); and c) leaflets were floated on aminotriazole solutions (12, 15, 18, 21 and 24 mg/dish) in the presence and absence of a fixed concentration of NH_4SCN (10.9 mg/dish - molar ratios of 1:1 to 2:1, aminotriazole: NH_4SCN).

4.2.1.2 Influence of various additives on bracken leaflet scorching by aminotriazole

Leaflets were floated on aminotriazole solutions (12 mg/dish) in the presence of a range of additive at a 1:1 molar ratio, except where otherwise stated.

4.2.1.3 Comparison of the influence of a thioacetamide and ammonium thiocyanate on scorching of bracken leaflets by aminotriazole

Two experiments were designed; a) leaflets were floated on aminotriazole solutions (12 mg/dish) in the presence of a range of concentrations of NH_4SCN and thioacetamide (5.4, 10.9, 21.7 and 32.6 mg/dish NH_4SCN and 5.4, 10.7, 21.4 and 32.1 mg/dish thioacetamide - molar ratios of 1:0.5 to 1:3 aminotriazole:additive); and b) leaflets were floated on aminotriazole solutions (3, 6 and 12 mg/dish) containing 5.4, 10.7 and 21.4 mg/dish of thioacetamide respectively (all 1:2 aminotriazole to thioacetamide molar ratios).

4.2.1.4 Influence of the degree of site exposure on scorching of bracken leaflets by aminotriazole

Bracken pinnules were collected from three sites varying in their degree of exposure, a) from the summit of a hill (200 m), b) from the base of the east facing slope of that hill in a sheltered hollow, c) from a nearby wooded area. The leaflets were floated on aminotriazole solutions (12 mg/dish) in the presence of a range of NH_4SCN concentrations (2.7, 5.4, 10.9 and 21.7 mg/dish - 1:0.25 to 1:2, aminotriazole: NH_4SCN)

4.2.1.5 Influence of ammonium thiocyanate on bracken leaflet scorching by asulam

Leaflets were floated on a 200 mg/dish asulam solution in the presence of a range of NH_4SCN concentrations (10.9, 21.7, 32.6, 43.4 and 86.9 mg/dish - molar ratios of 0.25:1 to 2:1, NH_4SCN :asulam. Assessment was made 120h after treatment.

4.2.1.6 Influence of ammonium thiocyanate and ammonium chloride on scorching of dock leaf discs by aminotriazole

Two experiments were designed; a) leaf discs were floated on aminotriazole solutions (0.6, 6 and 18 mg/dish) in the presence and absence of equimolar concentrations of NH_4SCN (0.5, 5.4 and 16.3 mg NH_4SCN); and b) leaf discs were floated on aminotriazole solutions (12 mg/dish) in the presence of a range of NH_4SCN (2.7, 5.4 and 10.9 mg/dish) and NH_4Cl (1.9, 3.8 and 7.6 mg/dish) concentrations - molar ratios of 0.25:1 to 1:1 additive to aminotriazole.

4.2.1.7 Influence of ammonium thiocyanate on scorching of potato leaf discs by aminotriazole

Leaf discs were floated on aminotriazole solutions (0.6, 6 and 18 mg/dish) in the presence and absence of equimolar concentrations of NH_4SCN (0.5, 5.4 and 16.3 mg NH_4SCN).

4.2.2 Bracken field trials

All field experiments were carried out at Carbeth, Stirling-shire (Grid Reference NS527798). Spraying was carried out using an ICI Mark 3 Knapsack sprayer at an output pressure of 30 p.s.i. As far as possible, or unless otherwise stated, spraying was done around the period of full frond development when systemic herbicides such as aminotriazole and asulam are most effective (Volger and Rösger, 1972; Soper, 1972).

4.2.2.1 Influence of ammonium and potassium thiocyanate combinations on bracken control by aminotriazole

In this trial the effectiveness of aminotriazole alone, aminotriazole: NH_4SCN formulations of molar ratios 1:0.5, 1:0.75, 1:1 and 1:1.25 and aminotriazole:KSCN (1:1) were assessed. KSCN was tested as an alternative to the NH_4 salt following bioassay results. Plot size was 7m x 7m with a 1m path between plots. A randomised block design with three replicates per treatment was employed. Aminotriazole was applied at a rate of 42g/plot (8.4kg/ha) at a volume rate of 2l/plot (420l/ha). An NH_4SCN control treatment of 38g/plot (equivalent to the NH_4SCN level used in the 1:1 molar ratio with aminotriazole) was included on an adjacent plot for scorching observations. Cook (1979) had already shown no effect on control by NH_4SCN when applied at a similar rate on this site. The plots were laid down on 15/6/79 when the bracken was 20-45 cm high. Spraying took place on 2/8/79. Observations on scorching by the treatments were made during the remainder of the season. Assessment of control was carried out in the three following seasons by counting the number of fronds in five 1m^2 quadrats from the central 25m^2 of each plot. The heights of fifteen fronds from the centre area were also recorded. Where appropriate analysis of the data was made by two-way Analysis of Variance with Duncans Multiple Range Test for difference between means.

4.2.2.2 The influence of spraying date and the presence of ammonium thiocyanate on bracken control by asulam

In this trial the effectiveness of asulam alone and in combination with NH_4SCN (1:10) on two spraying dates were assessed.

Two dates were tried to ascertain if NH_4SCN could broaden the narrow spraying date range where asulam is effective (Soper, 1972; Veerasekaran et al., 1976; 1977). Asulam was applied at a rate of 2.2kg/ha as Asulox (May and Baker Ltd.) which is below the recommended dosage rate of 4.4kg/ha (Anon., 1974). Although promising results have been achieved at 2.2kg/ha (Scragg et al., 1972), it was felt that at this lower rate differences between the asulam and asulam/ NH_4SCN formulations would be easier to observe. The NH_4SCN level employed was 7.6kg/ha which, although resulting in an asulam: NH_4SCN ratio of 1:10, is equivalent to the level used at the 1:1 molar ratio used in the aminotriazole trial(4.2.2.1). Experimental design and assessment were as for 4.2.2.1. The plots were laid down on 15/6/79 with three spraying dates being envisaged. Spraying took place on 5/7/79 and 3/8/79 as early and mid spraying dates. Throughout the remainder of August and the early part of September inclement weather prevented a third spraying date being possible. This serves to highlight the problem with the narrow optimum spray date range for systemic herbicides in bracken control.

4.2.2.3 Influence of thioacetamide on the control of bracken by aminotriazole

In this trial the effectiveness of aminotriazole alone and in combination with thioacetamide (1:2) were compared. Experimental design and assessment were as per 4.2.2.1 but with only data from two seasons following treatment being available. Aminotriazole was again applied at 42g/plot (8.4kg/ha), thioacetamide at 73.5g/plot (14.7kg/ha). The plots were laid down on 13/6/80 and sprayed on 1/8/80.

4.3 RESULTS

4.3.1 Herbicide scorch bioassays

4.3.1.1 Influence of ammonium thiocyanate on scorching of bracken leaflets by aminotriazole

From table 4.1a it can be seen that scorching was induced to the same extent by all concentrations of aminotriazole employed. NH_4SCN (1:1) decreased scorching at all levels compared with aminotriazole alone. Additionally, in the case of the aminotriazole: NH_4SCN combinations, scorching decreased as the concentration of NH_4SCN increased even though the ratio of aminotriazole to NH_4SCN remained constant.

When the aminotriazole concentration was fixed (6 mg/dish) and the concentration of NH_4SCN varied from 2.7 to 21.7 mg/dish, the degree of scorching decreased in a continuous trend as the concentration of NH_4SCN increased (table 4.1b), see also plate 4.1.

When the concentration of NH_4SCN was fixed (10.9 mg/dish) and the concentration of aminotriazole varied from 12 to 24 mg/dish, aminotriazole alone brought about the same degree of scorching in all cases and NH_4SCN decreased scorching to the same extent in all cases (table 4.1c).

Throughout these three experiments, controls containing NH_4SCN at all levels employed showed no effect on the leaflets.

Table 4.1 Influence of ammonium thiocyanate on scorching of bracken leaflets by aminotriazole

Treatment		Scorch rating ^a
a)	control	0
	aminotriazole (3mg)	5
	aminotriazole (3mg):NH ₄ SCN (2.7mg), 1:1	4
	aminotriazole (6mg)	5
	aminotriazole (6mg):NH ₄ SCN (5.4mg), 1:1	2
	aminotriazole (12mg)	5
	aminotriazole (12mg):NH ₄ SCN (10.9mg), 1:1	1
b)	control	0
	aminotriazole (6mg)	5
	aminotriazole (6mg):NH ₄ SCN (2.7mg), 1:0.5	4
	aminotriazole (6mg):NH ₄ SCN (5.4mg), 1:1	2
	aminotriazole (6mg):NH ₄ SCN (10.9mg), 1:2	1
	aminotriazole (6mg):NH ₄ SCN (21.4mg), 1:4	0
c)	control	0
	aminotriazole (12mg)	5
	aminotriazole (12mg):NH ₄ SCN (10.9mg), 1:1	1
	aminotriazole (15mg)	5
	aminotriazole (15mg):NH ₄ SCN (10.9mg), 1.25:1	1
	aminotriazole (18mg)	5
	aminotriazole (18mg):NH ₄ SCN (10.9mg), 1.50:1	1
	aminotriazole (21mg)	5
	aminotriazole (21mg):NH ₄ SCN (10.9mg), 1.75:1	1
	aminotriazole (24mg)	5
	aminotriazole (24mg):NH ₄ SCN (10.9mg), 2:1	1

^a Scale 0 = no scorching, 5 = complete scorching. Assessed after 72h. Treatment concentrations (in parentheses) are in mg/22 ml in each dish.



Plate 4.1 Bracken pinnule bioassay (72h assessment). Top left to bottom right: aminotriazole alone; aminotriazole + NH_4SCN (1:0.5); aminotriazole + NH_4SCN (1:1); aminotriazole + NH_4SCN (1:2); aminotriazole + NH_4SCN (1:4); control.



Plate 4.2 Bracken fronds - untreated control area.

4.3.1.2 Influence of various additives on bracken leaflet scorching by aminotriazole

Scorching was considerably reduced by all the SCN salts tested. The NH_4 and Ca salts appeared to be slightly more effective than the K and Na. KCN and $\text{K}_4\text{Fe}^{\text{II}}(\text{CN})_6$ also reduced scorching considerably. None of the above additives showed any great effect on the leaflets when applied in the absence of aminotriazole. $\text{K}_3\text{Fe}^{\text{III}}(\text{CN})_6$, both alone and in the presence of aminotriazole, brought about complete blackening of the leaflets. Of the range of halides tested, only NaF showed a marginal decrease in scorching. NaBr had no effect but NaI brought about considerable scorching in the absence of aminotriazole. Neither NH_4NO_3 nor NH_4 acetate had any influence on scorching. None of the phenols reduced aminotriazole scorching and only resorcinol had no effect in the absence of aminotriazole. The others all produced rapid blackening of the leaflets. Both thiourea and thioacetamide reduced aminotriazole scorching to some extent, and cysteine hydrochloride had only a marginal effect. Of these three, only thioacetamide had no effect on the leaflets when added alone (table 4.2).

4.3.1.3 Comparison of the influence of thioacetamide and ammonium thiocyanate on scorching of bracken leaflets by aminotriazole

When the concentration of aminotriazole was fixed at 12 mg/dish and the concentration of NH_4SCN varied from 5.4 to 32.6 mg/dish, the degree of scorching decreased as the concentration of NH_4SCN increased, except at the highest NH_4SCN level where the additive, if applied alone,

Table 4.2 Influence of various additives on bracken leaflet scorching
by aminotriazole

Additive	Scorch rating (combination)	Scorch rating (no aminotriazole)
none control	0	0
none aminotriazole	5	0
NH_4SCN	1	0
KSCN	2	0
NaSCN	2	0
$\text{Ca}(\text{SCN})_2^a$	0	0
$\text{Ca}(\text{SCN})_2^b$	1	0
KCN	2	1
$\text{K}_4\text{Fe}^{\text{II}}(\text{CN})_6$	2	1
$\text{K}_3\text{Fe}^{\text{III}}(\text{CN})_6$	5	5
NH_4NO_3	5	0
NH_4 acetate	5	1
NaI	5	3
NaBr	5	0
NaCl	5	0
NaF	4	1
phenol	5	5
catechol	5	4
resorcinol	5	0
quinol	5	4
thiourea	3	3
thioacetamide	3	0
cysteine hydrochloride	4	2

Scorch assessment was as per table 4.1. Aminotriazole concentration was 12mg/22ml in each dish. Additive concentrations were equimolar with aminotriazole.

^a aminotriazole:Ca(SCN)₂, 1:1

^b aminotriazole:SCN, 1:1

was beginning to have an effect. In comparison thioacetamide, when applied at the same molar concentration as NH_4SCN , had no effect in the absence of aminotriazole. This resulted in a continual decrease in scorching by the aminotriazole/thioacetamide combinations as the concentration of thioacetamide increased (table 4.3a).

In the second set of experiments, scorching was induced to the same extent by all three aminotriazole levels tested. Thioacetamide at twice the molar concentration decreased the scorching in all cases as compared to aminotriazole alone. For the combination of aminotriazole and thioacetamide, scorching decreased as the concentration of thioacetamide increased although the 1:2 to additive ratio remained constant (table 4.3b).

Throughout these experiments, thioacetamide applied alone had no effect at any level tested.

4.3.1.4 Influence of the degree of site exposure on scorching of bracken leaflets by aminotriazole

Aminotriazole scorching was induced to different degrees in bracken from the three sites (table 4.4). The scorch rating was highest in site a), the most exposed site, and lowest in site c), the shaded and sheltered site. For sites a) and b), NH_4SCN reduced scorching in an increasing trend, but at the highest level tested (21.7 mg, 1:2 aminotriazole to NH_4SCN) the scorch rating was the same as for the corresponding NH_4SCN control, indicating that the remaining scorch was due to NH_4SCN . For site c), scorching was reduced at the lowest NH_4SCN level (2.7 mg/dish, 1:0.25 aminotriazole to NH_4SCN), thereafter it began

Table 4.3 Comparison of the influence of thioacetamide and ammonium thiocyanate on scorching of bracken leaflets by aminotriazole

Treatment		Scorch rating (combination)	Scorch rating (no aminotriazole)
a) control			
aminotriazole (12 mg)		0	0
aminotriazole (12 mg):NH ₄ SCN(5.4mg),1:05		5	0
aminotriazole (12 mg):NH ₄ SCN(10.9mg),1:1		4	0
aminotriazole (12 mg):NH ₄ SCN(21.7mg),1:2		1	0
aminotriazole (12 mg):NH ₄ SCN(32.6mg),1:3		0	1
aminotriazole (12 mg):thioacetamide(5.4mg),1:0.5		2	2
aminotriazole (12 mg):thioacetamide(10.7mg),1:1		4	0
aminotriazole (12 mg):thioacetamide(21.4mg),1:2		1	0
aminotriazole (12 mg):thioacetamide(32.1mg),1:3		0	0
		0	0
b) control			
aminotriazole (3 mg)		0	
aminotriazole (3 mg):thioacetamide(5.4mg),1:2		5	
aminotriazole (6 mg)		3	
aminotriazole (6 mg):thioacetamide(10.7mg),1:2		5	
aminotriazole (12 mg)		0	
aminotriazole (12 mg):thioacetamide(21.4mg),1:2		5	
		0	

Scorch assessment and treatment concentrations are as table 4.1

Table 4.4 Influence of site exposure on scorching of bracken leaflets by aminotriazole/ammonium thiocyanate combinations

Site	Scorch rating ^a					
	aminotriazole:NH ₄ SCN ratio (M:M)					
	1:0	1:0.25	1:0.5	1:1	1:2	0:2
a)	10	9	7	3	1 ^b	1 ^b
b)	7	6	6	3	2 ^b	2 ^b
c)	2	1	3 ^b	3 ^b	4 ^b	4 ^b

^a Scale 0 = no scorching, 10 = complete scorching. Assessed after 72h.

Aminotriazole was at 12 mg/22 ml in each dish.

^b Scorching by the aminotriazole:NH₄SCN treatment the same as effect from the equivalent NH₄SCN control.

to increase. The control treatments indicated that this was due only to the NH_4SCN . The level of NH_4SCN scorch also varied with site exposure. In this case the scorch was highest in the shaded site c) and lowest in site a).

4.3.1.5 Influence of ammonium thiocyanate on bracken leaflet scorching by asulam

Preliminary experiments showed that 200 mg/dish of asulam was necessary to produce any scorch symptoms on bracken leaflets. At all the levels employed, NH_4SCN had no effect on the degree of scorching induced by the asulam. A good deal of scorching was observed from the higher concentrations of NH_4SCN when applied in the absence of asulam (table 4.5).

4.3.1.6 Influence of ammonium thiocyanate and ammonium chloride on scorching of dock leaf discs by aminotriazole

The effect of aminotriazole on dock leaf discs can be seen in table 4.6a. At all levels employed some degree of scorching resulted, the amount increasing as aminotriazole concentration increased. At the two higher levels of aminotriazole (6 and 18 mg/dish) equimolar NH_4SCN decreased the degree of scorching. When the concentration of aminotriazole was fixed (12 mg/dish) and the concentration of NH_4SCN varied from 2.7 to 10.9 mg/dish, the degree of scorching decreased as the concentration of NH_4SCN increased. When employed at similar molar concentrations as NH_4SCN , NH_4Cl had no effect on aminotriazole scorching. Some effect from NH_4Cl applied alone was noted (table 4.6b).

Table 4.5 Influence of ammonium thiocyanate on bracken leaflet
scorching by asulam

Treatment	Scorch rating (combination)	Scorch rating (no asulam)
control	0	0
Asulam (200 mg)	3	0
Asulam (200 mg):NH ₄ SCN (10.9mg), 1:0.25	3	0
Asulam (200 mg):NH ₄ SCN (21.7mg), 1:0.5	4	1
Asulam (200 mg):NH ₄ SCN (32.6mg), 1:0.75	5	2
Asulam (200 mg):NH ₄ SCN (43.4mg), 1:1	5	5
Asulam (200 mg):NH ₄ SCN (86.9mg), 1:2	5	5

Scorch assessment and treatment concentrations are as table 4.1 except
assessment was made after 120h.

Table 4.6 Influence of ammonium thiocyanate and ammonium chloride on scorching of dock leaf discs by aminotriazole

Treatment	Scorch rating (combination)	Scorch rating (no aminotriazole)
a) control	0	0
aminotriazole (0.6 mg)	1	0
aminotriazole (0.6 mg):NH ₄ SCN(0.5mg), 1:1	1	1
aminotriazole (6 mg)	2	0
aminotriazole (6 mg):NH ₄ SCN(5.4mg), 1:1	1	1
aminotriazole (18 mg)	5	0
aminotriazole (18 mg):NH ₄ SCN(16.2mg), 1:1	1	1
b) control	0	0
aminotriazole (12 mg)	5	0
aminotriazole (12 mg):NH ₄ SCN(2.7mg), 1:0.25	2	0
aminotriazole (12 mg):NH ₄ SCN(5.4mg), 1:0.5	1	0
aminotriazole (12 mg):NH ₄ SCN(10.9mg), 1:1	0	0
aminotriazole (12 mg):NH ₄ Cl(1.9mg), 1:0.25	5	1
aminotriazole (12 mg):NH ₄ Cl(3.8mg), 1:0.5	5	1
aminotriazole (12 mg):NH ₄ Cl(7.6mg), 1:1	5	2

^a Scorch assessment and treatment concentrations are as per table 4.1.

4.3.1.7 Influence of ammonium thiocyanate on potato leaf disc scorching by aminotriazole

At the lowest level employed (0.6 mg/dish), no effect from aminotriazole was noted. As the concentration of aminotriazole was raised, an increasing scorch of the leaf discs occurred. NH_4SCN , when applied alone, induced its own scorch at all levels used, the effect increasing as the concentration was raised. When applied as equimolar combinations, no decrease in aminotriazole scorch as a result of the presence of NH_4SCN could be observed (table 4.7).

Table 4.7 Influence of ammonium thiocyanate on scorching of potato leaf discs by aminotriazole

Treatment	Scorch rating	Scorch rating (no aminotriazole)
control	0	0
aminotriazole (0.6 mg)	0	0
aminotriazole (0.6 mg): NH_4SCN (0.5mg), 1:1	1	1
aminotriazole (6 mg)	2	0
aminotriazole (6 mg): NH_4SCN (5.4mg), 1:1	2	2
aminotriazole (18 mg)	4	0
aminotriazole (18 mg): NH_4SCN (16.3mg), 1:1	4	4

Scorch assessment and treatment concentrations are as for table 4.1.

4.3.2 Bracken field trials

4.3.2.1 Influence of ammonium and potassium thiocyanate combinations on bracken control by aminotriazole

Spraying was carried out on a warm dry day. No rain occurred for at least 6 days afterwards.

Visual inspection made 14 days after spraying revealed heavy scorching in these plots treated with aminotriazole alone. This scorching was reduced in all the SCN containing formulations, including KSCN, to be replaced by SCN scorching. SCN scorch is characteristically orange-rust coloured and tends to be confined to the marginal areas of the pinnules. Aminotriazole scorch is darker, generally more widespread over the pinnules and more severe in intensity than SCN scorching, everything being equal. Plates 4.2-4.6 are photographs of control and treated areas taken 14 days after treatment. Typical aminotriazole scorch is shown in plate 4.3. At the lowest NH_4SCN level (3.8 kg/ha, 1:0.5 aminotriazole to NH_4SCN) there was very little sign of aminotriazole or SCN scorching (plate 4.4). As the concentration of SCN employed increased, a trend towards increasing SCN scorch was observed, plates 4.4-4.6.

Observations made the following season revealed some effect on the grass sward with the aminotriazole alone and when with the highest NH_4SCN level (1:1.25, aminotriazole to NH_4SCN). There was considerable litter accumulation in the spring of this season on the aminotriazole alone plots. This may have affected early grass growth to some extent although direct contact of the spray with the sward through the



Plate 4.3 Bracken fronds sprayed 14 days previously with aminotriazole.



Plate 4.4 Bracken fronds sprayed 14 days previously with aminotriazole + NH_4SCN (1:0.5).



Plate 4.5 Bracken fronds sprayed 14 days previously with aminotriazole + NH_4SCN (1:1)



Plate 4.6 Bracken fronds sprayed 14 days previously with aminotriazole + NH_4SCN (1:1.25).

reasonably sparse frond cover is more likely. Litter accumulation on the SCN treated areas was no more than on control plots. By the following season all affected swards had recovered.

Frond density measurements made one year after spraying showed that 77% control by aminotriazole had been achieved (table 4.8). Control from all the aminotriazole/SCN formulations was significantly greater, in the range 94 to 97% control. In all cases where fronds emerged, they were stunted and generally chlorotic. Two years following treatment, control by all formulations was markedly less. Aminotriazole alone achieved 37% control. All aminotriazole combinations again achieved significantly greater control over aminotriazole alone. By the third season following treatment, aminotriazole alone showed no significant difference over the untreated. Although no significant difference was revealed between any of the aminotriazole/ NH_4SCN combinations or between the 1:1 aminotriazole/KSCN and 1:1 aminotriazole/ NH_4SCN treatments, aminotriazole: NH_4SCN (1:0.5) was the only combination which gave significantly greater control than aminotriazole alone (table 4.8). Frond height measurements made over the three seasons following treatment revealed no significant differences between any aminotriazole treatment while they were all reduced compared to the control treatment (table 4.9).

Table 4.8 Influence of ammonium and potassium thiocyanate combinations on bracken control by aminotriazole (8.4 kg/ha) - frond density measurements

Treatment	MFN *		
	years after treatment		
	1	2	3
control	a ₄₃	a ₄₁	a ₃₅
aminotriazole alone	b ₁₀ (77)	b ₂₆ (33)	ba ₂₆ (26)
aminotriazole:NH ₄ SCN,1:0.5	c ₂ (95)	c ₉ (78)	c ₉ (74)
aminotriazole:NH ₄ SCN,1:0.75	c ₂ (95)	c ₁₃ (68)	cb ₁₆ (54)
aminotriazole:NH ₄ SCN,1:1	c ₂ (95)	c ₁₂ (71)	cb ₁₈ (49)
aminotriazole:NH ₄ SCN,1:1.25	c ₂ (95)	c ₁₆ (61)	cb ₁₉ (46)
aminotriazole:KSCN,1:1	c ₂ (95)	c ₁₆ (61)	cb ₁₉ (46)
LSD **	7	9	12

* mean numbers of fronds/m²

** least significant difference at the 5% level.

Figures in parentheses indicate % reduction in frond density over the control.

Values with a similar letter within each column are not significantly different in Duncans Multiple Range Test.

Table 4.9 Influence of ammonium and potassium thiocyanate combinations on bracken control by aminotriazole (8.4kg/ha)-frond height measurements

Treatment	MFH *		
	years after treatment		
	1	2	3
Control	a ₁₃₀	a ₁₂₉	a ₁₂₉
aminotriazole alone	b _{70 (46)}	b _{81 (37)}	b _{87 (33)}
aminotriazole:NH ₄ SCN,1:0.5	b _{41 (68)}	b _{52 (60)}	b _{57 (56)}
aminotriazole:NH ₄ SCN,1:0.75	b _{45 (65)}	b _{55 (57)}	b _{63 (51)}
aminotriazole:NH ₄ SCN,1:1	b _{36 (72)}	b _{46 (64)}	b _{49 (62)}
aminotriazole:NH ₄ SCN,1:1.25	b _{47 (64)}	b _{58 (55)}	b _{76 (41)}
aminotriazole:KSCN,1:1	b _{44 (66)}	b _{65 (50)}	b _{71 (45)}
LSD	32	38	36

* mean frond heights in cm.

Figures in parentheses indicate % reduction in frond height over control. All other details are as table 4.8.

4.3.2.2 Influence of spraying date and the presence of ammonium thiocyanate on bracken control by asulam

Spraying was carried out on 2 dates; 5/7/79 when the fronds were not fully unfurled and 3/8/79 when the full frond was available to the spray. On each date no rain occurred within at least 3 days afterwards.

Observations made 14 days after the first spraying date revealed considerable chlorosis of the pinnae, accompanied by blackening of their stems, in the asulam alone treatments (plate 4.7). These effects were restricted to the new growth, i.e., that which unfurled after spraying. With the asulam/ NH_4SCN (1:1) combination, there was no effect on the fronds other than some localised SCN scorching, again on the new growth (plate 4.8). No chlorosis or scorch was observed on asulam alone treated plots 14 days after their treatment on the second spraying date. The asulam/ NH_4SCN treated plots showed SCN type scorching similar to that observed above for NH_4SCN alone (4.3.2.1). In the year following treatment little effect on the grass sward, through litter accumulation or herbicide run off, was noted for any treatment.

One year after spraying, all treatments significantly reduced frond density (table 4.10). No significant differences between asulam alone and asulam/ NH_4SCN or between similar treatments on different spraying dates were revealed. Two years following application, all treatments still significantly reduced frond density. Asulam alone resulted in significantly better control than the asulam/ NH_4SCN combination when sprayed on the early date. No such difference occurred between the later spraying date treatments. There was again no difference



Plate 4.7 Bracken fronds sprayed 14 days previously with asulam (in early July) at 2.2 kg/ha.



Plate 4.8 Bracken fronds sprayed 14 days previously with asulam (2.2 kg/ha) + NH_4SCN (1:10) in early July.

Table 4.10 The influence of spraying date and the presence of ammonium thiocyanate on the control of bracken by asulam (2.2 kg/ha)-frond density measurements

Treatment	MFN		
	years after treatment		
	1	2	3
control	a ₃₀	a ₃₇	a ₃₁
asulam alone ^o	b ₂ (95)	d ₄ (89)	c ₈ (74)
asulam:NH ₄ SCN, 1:10 ^o	b ₁₀ (67)	b ₂₄ (35)	a ₃₀ (3)
asulam alone ^{oo}	b ₄ (85)	dc ₉ (76)	cb ₁₅ (52)
asulam:NH ₄ SCN, 1:10 ^{oo}	b ₈ (72)	cb ₁₈ (51)	ba ₂₅ (19)
LSD	10	12	12

^o spraying date early - 5/7/79

^{oo} spraying date mid - 3/8/79

all other details are as for table 4.8

between similar treatments on different spraying dates. One year later, only the asulam treatments without NH_4SCN gave significant control. Again at the early spraying date, asulam alone gave significantly greater control than the combination (table 4.10). Frond height measurements gave similar trends to the density data (table 4.11).

4.3.2.3 Influence of thioacetamide on the control of bracken by aminotriazole

Spraying was carried out on 1/8/80, a bright day. Unfortunately, a rain shower occurred 3 hours after spraying which may have resulted in some loss of chemical from the treated fronds.

Visual inspection 14 days after treatment revealed typical aminotriazole scorching on all aminotriazole treated plots, including those with thioacetamide included. Thioacetamide alone had no effect on the fronds. Heavier than normal litter accumulation and grass check (see 4.3.2.1) were noted for all aminotriazole treatments in the year following application although subsequent recovery in grass growth occurred.

Assessment made 1 year following spraying revealed that although both aminotriazole treatments significantly reduced frond densities, there was no significant difference between them. Thioacetamide alone had no effect. Two years following treatment there was no effect from any treatment (table 4.12). Frond height measurements gave similar trends although 2 years after spraying both aminotriazole treatments were significantly shorter than the control (table 4.12).

Table 4.11 The influence of spraying date and the presence of ammonium thiocyanate on the control of bracken by asulam (2.2kg/ha) - frond height measurements

Treatment	years after treatment		
	1	2	3
control	^a 108	^a 128	^a 132
asulam alone	^b 67 (38)	^c 70 (45)	^b 72 (45)
asulam:NH ₄ SCN,1:10	^a 82 (24)	^{ab} 104 (19)	^a 121 (8)
asulam alone	^b 61 (44)	^c 68 (47)	^b 77 (42)
asulam:NH ₄ SCN,1:10	^b 69 (36)	^{bc} 92 (28)	^a 108 (18)
LSD	29	25	26

All details are as table 4.9

Table 4.12 Influence of thioacetamide on the control of bracken by
aminotriazole (8.4 kg/ha)

Treatment	MFN		MFH	
	years after treatment			
	1	2	1	2
control	a ₃₉	a ₃₆	a ₁₃₀	a ₁₄₀
aminotriazole alone	b _{12 (69)}	a _{29 (20)}	b _{48 (63)}	b _{88 (37)}
aminotriazole:thioacetamide,1:2	b _{9 (76)}	a _{27 (25)}	b _{54 (58)}	b _{85 (39)}
thioacetamide alone	a _{39 (0)}	a _{33 (8)}	a _{129 (1)}	ba _{119 (15)}
LSD	14	16	32	37

All details are as for tables 4.8 and 4.9

4.4 DISCUSSION

From the experimental evidence obtained in the bioassays, it can be seen that NH_4SCN is capable of reducing aminotriazole scorching of bracken. The results suggest that the 1:1 molar ratio used commercially is not the optimum for all conditions. It would appear that the concentration of NH_4SCN was the critical factor determining the degree of aminotriazole scorch, no matter how much aminotriazole is present. As previously proposed by Cook and Duncan (1979), the important relationship may be that of the concentration of NH_4SCN relative to the ability of the plant to generate aminotriazole free radicals. ^{It is likely that} the plant has the capacity to bring about the formation of only a finite number of aminotriazole radicals and, within certain limits, the relative effects could be independent of aminotriazole concentration.

When applied in the field to bracken, the findings of Cook (1979) are confirmed in that the less aminotriazole scorching which takes place, the more the control. The results indicate that 3.8kg/ha of NH_4SCN (aminotriazole: NH_4SCN , 1:0.5) is sufficient to eliminate aminotriazole scorching without any NH_4SCN scorching, the degree of control being at least as good, if not better, than the 1:1 ratio. By three years after spraying the 1:0.5 combination was the only combination which gave significantly better control than aminotriazole alone. Longer term assessment would be necessary to verify this point. Although the degree of control from the 1:0.5 combination tested in the field was not statistically better than the 1:1 mixture, it is possible that because the degree of control was so great that the rate of

application of the aminotriazole may have been beyond the optimum for this site. Formulations such as 1:0.5 might produce better control than 1:1 mixtures at a reduced aminotriazole application rate. The NH_4SCN rate would stay at 3.8 kg/ha. The observations on the effect on the grass sward also make the lowest SCN level attractive compared to higher SCN levels.

As the results of table 4.4 suggest, the level of NH_4SCN sufficient to eliminate aminotriazole scorch may vary from site to site. The source of material for the bioassay was from the same site as the field trials took place. This site was on the west facing slope of the hill referred to in 4.2.1.4 and is a well exposed area. This could imply that the amount of SCN required on other sites is unlikely to be much greater and could be considerably less, although every site is different and may require a screen such as the present bioassay prior to recommendations for dosage rates and formulation type to be made. The factors governing the differential response on varying sites are not clear; however, other factors such as stage of maturity being equal, light intensity and nature (particularly uv content), and how it affects plant constituents which inhibit the biochemical processes involved in aminotriazole detoxification, may be involved.

Variations in the success of aminotriazole in bracken control have been observed. Kirkwood and Fletcher (1961), in the West of Scotland, observed only a 50% reduction in frond density using aminotriazole alone at 20 lb/acre. The activated formulation was even less effective. Erskine (1960), in the East of Scotland, and Hodgson (1960) in England had consistently better results with aminotriazole,

frond numbers decreases of more than 90% being achieved in the year after spraying and long term control resulting. Erskine (1968), summarising field trials carried out between 1958 and 1968, concluded for aminotriazole that rates of 5 and 7.5 lb/acre (5.6 and 8.4 kg/ha) produced long lasting control entirely acceptable in practice. Rain or drizzle immediately after spraying has been put forward as being important in the failure of aminotriazole in bracken control in some cases (Holroyd *et al.*, 1970). Brown and McKenzie (1972) have proposed that the poorer results in western UK were due to the wetter climate. Kirkwood and Fletcher (1961) concluded their poor results were due to late spraying. Uptake of asulam into bracken fronds has been shown to decrease with increasing frond age (Veerasekaran *et al.*, 1976; 1977). Lateness of spraying has also resulted in scorching by aminotriazole which was much less severe than that observed in previous applications at the optimum spraying time (Cook, 1979). This was perhaps due to less light affecting free radical formation. The reduced scorching did^{not} result in the increase in control the following season that one would expect with less aminotriazole detoxification. Basipetal translocation would still be likely (Veerasekaran *et al.*, 1977). This could indicate that lack of uptake is still an important factor in the poor performance of late season applied aminotriazole and illustrates the importance of which surfactants and other additives, including SCN, may have in improving efficiency of uptake and translocation when spraying takes place outwith the narrow optimum time period. The present work has shown that the variation in success of activated aminotriazole in bracken control may have been due, at least in part,

to variation in the free radical generating capacity of the bracken at the different sites. This would result in a differing ability to detoxify aminotriazole and so differing optimal requirements for aminotriazole and NH_4SCN .

The choice of NH_4SCN level for formulations is also important when one considers its increasing phytotoxic response in both bracken and the grass sward below as the NH_4SCN level is increased. The addition of diquat to activated aminotriazole formulations has been shown to result in rapid scorching which ultimately reduced effectiveness of control (Arbonnier, 1964; Varlet et al., 1964). The high rates of aminotriazole, hence NH_4SCN , used by Kirkwood and Fletcher (1961) would probably have resulted in a high degree of SCN scorch which may explain their poorer results with the activated formulations. When used in the bracken leaflet bioassay as an additive with aminotriazole, I resulted in a rapid black scorch. This effect also occurred in the field, a significant reduction in control by the combination compared with aminotriazole alone resulting (Cook et al., (1982). Thus, a high level of SCN scorch in the year of spraying may result in reduced control by aminotriazole in subsequent seasons. Considerably more SCN scorch was observed in the 1:1.25 aminotriazole: NH_4SCN formulation than in the 1:0.5 mixture. As already discussed, non-significantly better control from the 1:0.5 combination was observed. The level of SCN scorch of bracken leaflets also varied with the degree of site exposure. The reasons for this are again unclear. In an attempt to gain more understanding here, study of the more general herbicidal properties of SCN and its more phytotoxic

halide relative I, was made. The results of this are reported in chapters 5 and 6. Effects on the grass sward by NH_4SCN were noted at the higher combinations in the field trials; obviously, a minimal influence on the grass sward will be desirable for post-spraying management of bracken infested land if no re-seeding programme is carried out.

Other alternatives to SCN were tested in the bioassay for possible scorch reducing properties and low phytotoxicity. Of the additives tested only $\text{Ca}(\text{SCN})_2$ was more effective than the NH_4 salt. However when it was tested at equimolar SCN to aminotriazole, its effects were similar to that of the NH_4 salt. The NH_4 and Ca salts appeared to be slightly more effective than K and Na salts. This effect, however, would require further verification. In bracken, Varlet *et al.* (1964) replaced NH_4SCN with NaSCN as activator, this yielded poorer results the following season. In the bracken field trials of the present study, KSCN (slightly poorer to NH_4SCN in the bioassay) was no different from NH_4SCN as an additive with aminotriazole when used at equimolar concentrations. In the bioassay, SCN, CN and $\text{Fe}^{\text{II}}(\text{CN})_6$ all behaved similarly in reducing aminotriazole scorching. All three have been shown to exhibit free radical inhibition properties *in-vitro* (Treinin and Hayon, 1976; Cook and Duncan, 1979). For toxicological reasons CN and $\text{Fe}^{\text{II}}(\text{CN})_6$ were not considered worthy of further study. Carter (1975) demonstrated reduced 3-ATAL formation with aminotriazole in the presence of thiourea, thioacetamide and cysteine. These results are reflected in the reduced scorching found in the present study. It should be noted that no additive tested was

better than NH_4SCN , which was the only one, with the exception of thioacetamide, tested over a range of concentrations. Time did not permit a more extensive study using all the promising additives, thus the possibility cannot be ruled out that some of these may show improved effects at different concentrations.

Because of its ability to reduce aminotriazole scorch, and the absence of any effect on bracken leaflets when applied alone, it was decided to look further at the possibility of using thioacetamide in aminotriazole formulations. In the bioassay, when aminotriazole: additive ratios of 1:0.5 to 1:3 were used, comparison of NH_4SCN and thioacetamide as additives showed that both were equally effective in reducing scorching. At the higher level of additive, thioacetamide had the advantage of not inducing its own phytotoxic effect. Further experiments also indicated that, as found for NH_4SCN , it was the thioacetamide level which was the important factor in reducing aminotriazole scorching. With promising possibilities, it was decided to apply thioacetamide in bracken control trials with aminotriazole. A 1:2 molar ratio of aminotriazole to thioacetamide was chosen for high scorch reduction potential without additive scorch. As the results have shown (table 4.12) the aminotriazole/thioacetamide formulations were disappointing in the field trials. Virtually no difference was observed between the aminotriazole formulations, with and without additive, in terms of scorching and control. The rapid regeneration observed two years after application with aminotriazole suggests that the rain which followed spraying may have resulted in a reduced level of chemical being retained and absorbed, leading subsequently to poor

control. Holroyd et al. (1970) also observed this. Thioacetamide appears to have done little to improve matters. It should be said that the thioacetamide may not have been taken up by the fronds very effectively. Further work would be necessary to evaluate fully the potential of thioacetamide in aminotriazole bracken control formulations.

When asulam was incorporated into the bioassay, it was found that relatively high concentrations in comparison to aminotriazole were necessary before much scorching occurred. No NH_4SCN level was successful in reducing the scorch at the level of asulam employed. More work would be necessary to fully investigate this aspect in the bioassay situation. Despite not having attained any advantage by NH_4SCN in the asulam bioassay, it was decided to press on and gauge its effect in the field situation. The initial observations after the early spraying were encouraging, SCN reducing asulam's phytotoxic effects.

Veerasekaran et al. (1977) also observed chlorosis and scorch in young fronds sprayed with asulam. It would appear that the translocation was mainly acropetal at the time of spraying, when the fronds were not fully expanded (Veerasekaran et al., 1977). This would result in a concentrating of asulam in the frond tips which could account for the observed phytotoxic responses only being seen in the newly unfurled top growth. Although NH_4SCN prevented these effects, any benefit was not observed in terms of control, indeed a deleterious effect on long term control has resulted from the treatment (table 4.10). The lack of any scorching response after the mid season spraying date is not surprising. Holroyd and Thompson (1978), in summarising a series of experiments using asulam on different sites, state that asulam treatments rarely

produce any herbicidal symptoms on the bracken foliage in the season of treatment, the main effects normally being apparent in subsequent seasons. The conclusion one could draw from the asulam experiments is that the oxidative conjugation via a free radical is not a major detoxification process of asulam in bracken. However, the level of NH_4SCN was chosen as the same level known to be effective in reducing aminotriazole scorch, i.e., around the optimum level of NH_4SCN thought to be able to cope with the aminotriazole free radical generating capacity of the bracken. The possibility exists that the oxidative potential of asulam is lower compared to aminotriazole, due to it forming a less stable free radical intermediate or some other factor. In this way the level of NH_4SCN employed may have been in excess of that required by the effective free radical generating capacity of the bracken in terms of asulam. As the level of asulam (2.2 kg/ha) employed was also below the recommended dosage rate, further studies, using higher asulam rates and lower concentrations of NH_4SCN than used here, may result in improved control.

The results on the influence of NH_4SCN on scorching of dock leaf discs by aminotriazole are encouraging. A similar scorching by aminotriazole to that found for bracken leaflets was observed. This was not a salt effect, as shown by the absence of scorch reducing ability by using NH_4Cl . Once again it appeared that the level of SCN was the critical factor in determining the degree of aminotriazole scorch. It thus seems possible that the same arguments as for bracken will apply in that different stands may vary in their free radical generating capacity and thus aminotriazole/ NH_4SCN formulations will require careful selection for optimal systemic activity.

The level of NH_4SCN used in the potato leaf disc bioassay appears to be too high, in that its own phytotoxic response masked any beneficial effect of reducing aminotriazole scorch. Again, a good deal of further study would be necessary to optimise aminotriazole/ NH_4SCN combinations for groundkeeper control.

The necessity to use field grown bracken leaflets as the bioassay material puts restriction on the time periods when the bioassay can be utilised. Only leaflets gathered between the time of full unfurlment of the fronds and before any sign of senescence were used since the scorching effects began to vary outwith these times. This, like the field spraying, restricts the bioassay to 4 to 6 weeks in mid summer. It is not weather dependent and since each assay takes only 3-4 days, a good deal can be covered over the full period. It can be advantageous to be working with the target weed you are ultimately aiming to control. The scorch produced in the bioassay by both aminotriazole and NH_4SCN bore close resemblance to that found in the field trials. The use of dwarf bean (see 4.1) may have resulted in differential phytotoxic responses from herbicide or additive being produced which might have led to erroneous deductions being made regarding bracken. The same argument may be made for the dock and groundkeeper potato situation.

The study has shown that perhaps the value of activated aminotriazole on bracken is underrated. If bracken stands were treated singly, as far as aminotriazole scorching and the amount of SCN required to cope with the free radical generating capacity was concerned, more consistently good control could result. Because of the possible

effects on the grass sward below, the density of the frond cover need also be taken into account if considering activated aminotriazole on bracken. Manipulation of NH_4SCN level would also be of benefit in keeping sward damage to a minimum. Aminotriazole has potential uses in old sward destruction and stubble cleaning (Joice and Norris, 1962; Fryer and Makepeace, 1978). In these situations, since mixed weed populations will be present, the tailoring of aminotriazole/ NH_4SCN formulations may not be quite so relevant as in bracken, unless particular problems of dock, groundkeeper potatoes or perhaps, couch grass, were being faced.

CHAPTER 5

STUDIES IN THE SELECTIVE HERBICIDAL ACTIVITY OF IODIDE

5.1 INTRODUCTION

The previous chapter has indicated the potential of thiocyanate and its halide relative iodide as additives for improving the efficiency of translocation and performance of systemic herbicides, not only on bracken but on a range of target species. When used on bracken, the level of SCN scorch varied with the degree of site exposure, the reasons for this being unclear. I was toxic to bracken in both bioassay and field trials. In an attempt to gain more understanding here, as an initial step in the further development of these two ions as additives, attention was turned to their behaviour when applied to plants in their own right. These studies revealed some interesting aspects which are presented here and in the following chapter. Two chapters were taken merely for ease of presentation.

I may be applied in its own right to plants (Wain et al., 1966), as an additive in the formulation of other herbicides or be liberated within the plant by the metabolism of I containing herbicides, e.g. Ioxynil (Wain, 1964; Zaki et al., 1967). At low levels I is considered to be a micronutrient necessary for the healthy growth of plants (Lehr et al., 1958). Toxicity at higher levels has been recognised since as far back as 1813 (Anon., 1950). Only relatively recently has it been shown that the toxic dose varies so that there is a degree of selectivity between species (Wain et al., 1966). They showed that NaI

and KI, when applied through root or shoot, completely destroyed some plant species at certain concentrations, yet had little effect on others. Dwarf bean were particularly susceptible as were annual nettle, fat hen and all the legumes tested. Brassica spp. were very resistant to I. Umaly and Poel (1970) found different levels of sensitivity to I between barley, tomato and pea. Similar selective responses with herbicidally active iodobenzoic acids has also been demonstrated (Chamberlain and Wain, 1971).

The oxidation of I by peroxidases is well recognised (Saunders et al., 1964; Morrison and Bayse, 1973; Morrison and Schonbaum, 1976), the occurrence of such peroxidase enzymes being widespread in plants (Saunders et al., 1964). It has been proposed that I toxicity depends on its intracellular oxidation within the plant to molecular iodine (I_2) via its peroxidase enzyme system (Wain et al., 1966; Mynett and Wain, 1971, 1973), I_2 being considerably more toxic (Mynett and Wain, 1973). Differences in the relative activities of plant peroxidases in terms of their oxidation of I, in addition to the extent to which I can be accumulated in the leaves, have been proposed as possible mechanisms for the selectivity of applied I (Mynett and Wain, 1971). Although some peroxidase activity in I resistant tomatoes was demonstrated using phenolic substrates, Mynett and Wain (1971) could obtain no evidence of I-using peroxidases. They considered this was why this species could tolerate high levels of I. However, the ability of peroxidase to catalyse I oxidation is considered ubiquitous among peroxidases (Morrison and Bayse, 1973). This being the case, selectivity may be the result of the presence or absence of inhibitory substances affecting the oxidation to I_2 .

In mammals, as far as is known, the entire functional significance of I_2 is accounted for by its presence in the thyroid hormones (Underwood, 1977), I being readily oxidised within this tissue by an I specific peroxidase (Hosoya and Morrison, 1967). SCN is one of a small number of goitrogens which acts by inhibiting the concentration of I by the thyroid and the iodination of tyrosine in this tissue (Wolff et al., 1946; Raben, 1949) presumably through the inhibition of thyroid peroxidase (Maloof and Soodak, 1964). The overall action is reversible by I_2 supplementation (Underwood, 1977). The SCN ion is one of the main factors implicated in the goitrogenic effect of the brassica family (Barker, 1936; Gmelin and Virtanen, 1960; Langer, 1966; Paxman and Hill, 1974). It is present in the plant mainly in the form of glucosinolates, also called thioglucosides, although a small percentage has been shown to exist as free SCN (Paxman and Hill, 1974a). The enzyme myrosinase (EC 3.2.3.1), or thioglucosidase, which hydrolyses these glucosinolates is apparently unavailable in the plant unless cell damage occurs (Van etten et al., 1969) whereupon isothiocyanates are produced. Further mild hydrolysis yields SCN (Gmelin and Virtanen, 1960; Appelqvist and Josefsson, 1967; Van etten et al., 1969; Paxman and Hill, 1974a). Areas of cell collapse have been demonstrated, even in resistant plant species, in response to I applications (Mynett and Wain, 1971).

It would thus seem possible that the presence or production of differential SCN concentrations in response to I applications could well be a further selectivity mechanism by preventing the formation of I_2 by peroxidase enzymes.

The principal aims of this investigation were, initially, as follows:

- (a) a study of the effect of SCN on the in-vitro oxidation of I catalysed by peroxidase,
- (b) in-vivo studies to determine the potential SCN concentrations of a range of plant species and to compare this with their susceptibility to application of I.

These investigations are described below in section 5.A. Further studies, carried out in the light of these investigations, are covered in sections 5.B-D.

5.A The role of thiocyanate in the selective toxicity of iodide

5.A.1 Experimental

5.A.1.1 Materials

Horse-radish peroxidase (HRPO)-type 1, RZ = 0.6 - was purchased from the Sigma Chemical Company, London. H_2O_2 was of Aristar grade and was purchased from British Drug Houses, Ltd. All other reagents were of the purest grades available and were purchased from either British Drug Houses Ltd. or Hopkin and Williams Ltd.

5.A.1.2 Methods

5.A.1.2.1 HRPO catalysed oxidation of iodide by hydrogen peroxide

The rate of I oxidation by HRPO was determined by following the rate of formation of the triiodide ion (hereafter referred to as I_3) at

353 nm, as used by Mynett and Wain (1971). The reaction mixture contained as follows: 0.5 ml of 10 mM NaI, 2.5 ml of 0.04M phosphate buffer at pH 6.0, 0.1 ml of 0.5 mg/ml HRPO and 0.5 ml of water. Equilibration was for 10 min at 37°C in a 1 cm quartz cell situated in a Pye-Unicam SP1800 Spectrophotometer fitted with a temperature controlled block. The reaction was initiated by the addition of 0.1 ml of 10 mM H_2O_2 , this solution being prepared fresh every 5h. The change in absorbance, measured against a reagent blank, was continually monitored for at least 5 min. Controls with no HRPO present were also included to check for chemical oxidation. A small preliminary experiment was carried out to determine which of the factors were limiting under the above reaction conditions. These, and all subsequent oxidation experiments, were carried out at least twice to check that trends were consistent.

5.A.1.2.2 Influence of thiocyanate on the HRPO catalysed oxidation of iodide by hydrogen peroxide

The oxidation of I was carried out as per 5.A.1.2.1 with, in place of water, 0.5 ml of a range (0.02, 0.4, 1.32, 5 and 10 mM of NH_4SCN concentrations. To check that the effects were due to SCN, the influence of NH_4SCN (5mM) on I oxidation was compared to that of NaSCN and $(\text{NH}_4)_2\text{SO}_4$ (both 5mM).

5.A.1.2.3 Investigation into the thiocyanate content of various test plants

Dwarf French bean (Phaseolus vulgaris c.v. Masterpiece), pea (Pisum sativum c.v. Meteor), cabbage (Brassica oleracea c.v. January King)

and kale (c.v. Thousand Head, derived from Brassica oleracea) were sown into seed trays containing Fisons Levington sowing compost and maintained in a glasshouse at $20 \pm 2^{\circ}\text{C}$ under artificial lighting with a 16 h day length. Plant material was harvested after 4 weeks. The potential SCN concentration in the leaves was determined by a modification of the method of Johnston and Jones (1966), where, to obtain maximum enzymatic release of SCN from its precursors, the homogenised leaf tissue was incubated in a sealed glass flask for 16h at 37°C prior to refluxing. The final extract was also concentrated by a factor of six. The methods of Paxman and Hill (1974a) and Godsen (1978) were tested before the above procedure was adopted. These methods were discarded due to very low or non-measurable levels of SCN being manifested which did not conform to published data (Paxman and Hill, 1974, 1974a; Michajlovski, 1964).

5.A.1.2.4 Selective toxicity of I to the test plants

Similar 4 week old plants to those used in 5.A.1.2.3 were employed to determine their susceptibility to I application. After washing the roots thoroughly, one each of the four species was transplanted into a 10 cm pot (without drainage holes) containing vermiculite. A range of NaI concentrations (0, 1.5, 15, 75 and 150mg/pot) were added to the vermiculite in 150 ml deionised water. Four replicate pots per I level were employed. The pots were maintained under the same environmental conditions as described above. Watering with tap water was carried out as necessary. The effects of I were visually assessed after 24, 72, 120 and 168h on an arbitrary 0 to 5 scale depending on the degree of toxicity (0 = no effect, 5 = severe wilting, dessication or death).

5.A.2 Results

5.A.2.1 HRPO catalysed oxidation of iodide by hydrogen peroxide

The rate of I oxidation by HRPO under the standard reaction conditions (5.A.1.2.1) is shown in figure 5.1a. The rate of formation of I_3 rose steadily at approximately 0.85 abs. units/min, slowed down slightly, before an abrupt halt in the reaction after 145 sec. If only 0.05 ml of H_2O_2 was used then the rate of reaction was identical but the reaction finished after 70 sec at an I_3 reading of 50% of the former (figure 5.1b). Stepwise additions of 3 x 0.025 ml of H_2O_2 brought the reaction to an I limiting condition whereby any further addition of H_2O_2 gave no further I oxidation (preliminary experiments had shown HRPO to be well in excess). Thus, with the standard reaction conditions I was in excess. The oxidation tailed off slightly after 85 sec due to the reaction tending to an I and H_2O_2 limiting situation before all peroxide was eventually exhausted and the reaction stopped (figure 5.1a). If 0.125 ml of H_2O_2 was used initially then all the I was oxidised first and the reaction also stopped.

Figure 5.1c shows the oxidation of I in the absence of the enzyme. This chemical oxidation was considered as negligible.

5.A.2.2 Influence of thiocyanate of the HRPO catalysed oxidation of iodide by hydrogen peroxide

The results of the effect of SCN on the rate of I oxidation are shown in figure 5.2. The rate of formation of I_3 was decreased by all levels of SCN tested. At 10 mM the reaction was almost totally inhibited whilst at the lowest concentration (0.02mM) only a marginal reduction was

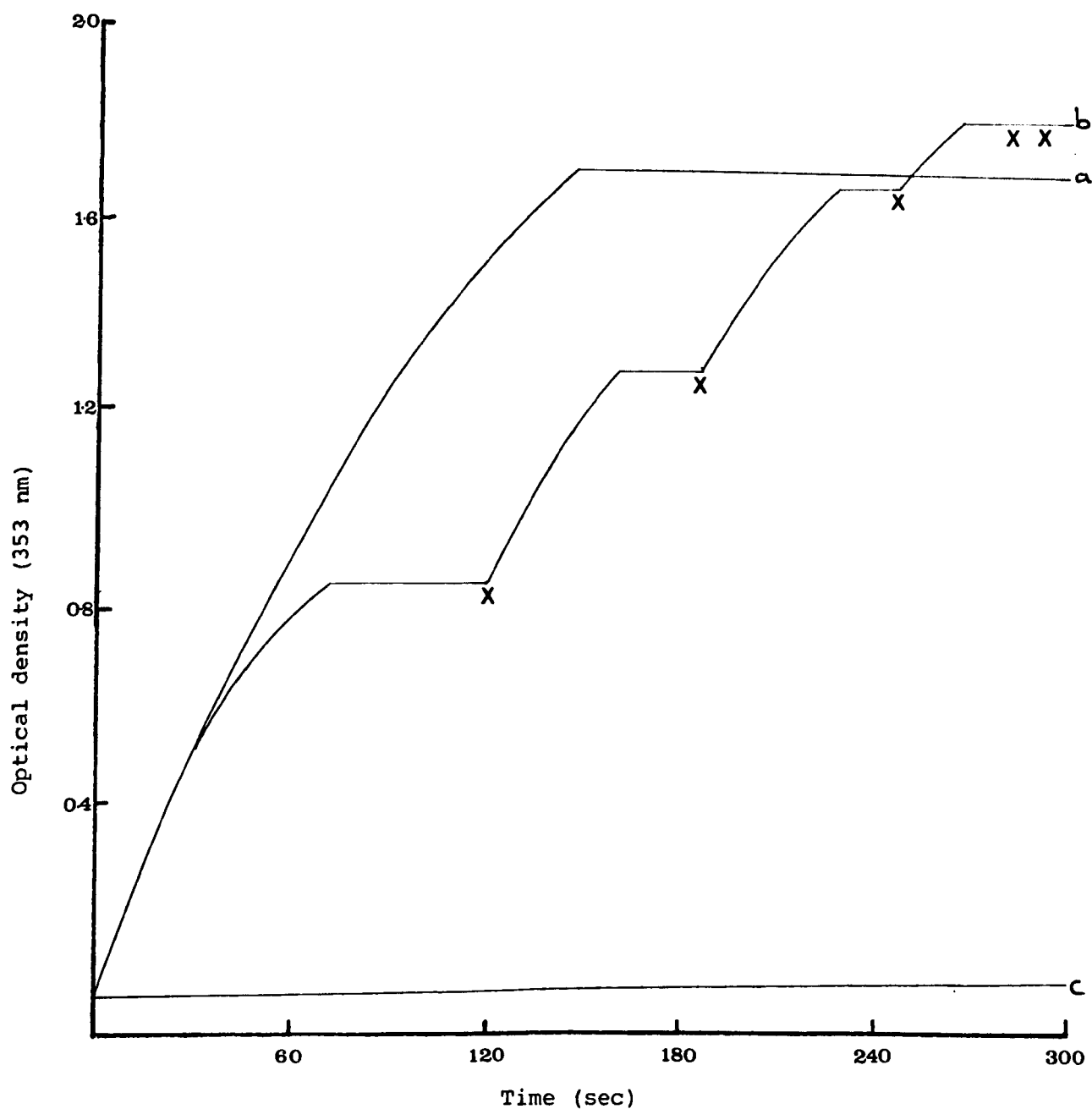


Figure 5.1 Horseradish peroxidase (HRPO) catalysed oxidation of I by H_2O_2 . a - I oxidation using $100\mu l$ of H_2O_2 under standard reaction conditions (5.A.1.2.1). b - I oxidation using $50\mu l$ H_2O_2 initially, followed by additions of $25\mu l$ H_2O_2 at every X. c - Standard reaction conditions using no HRPO.

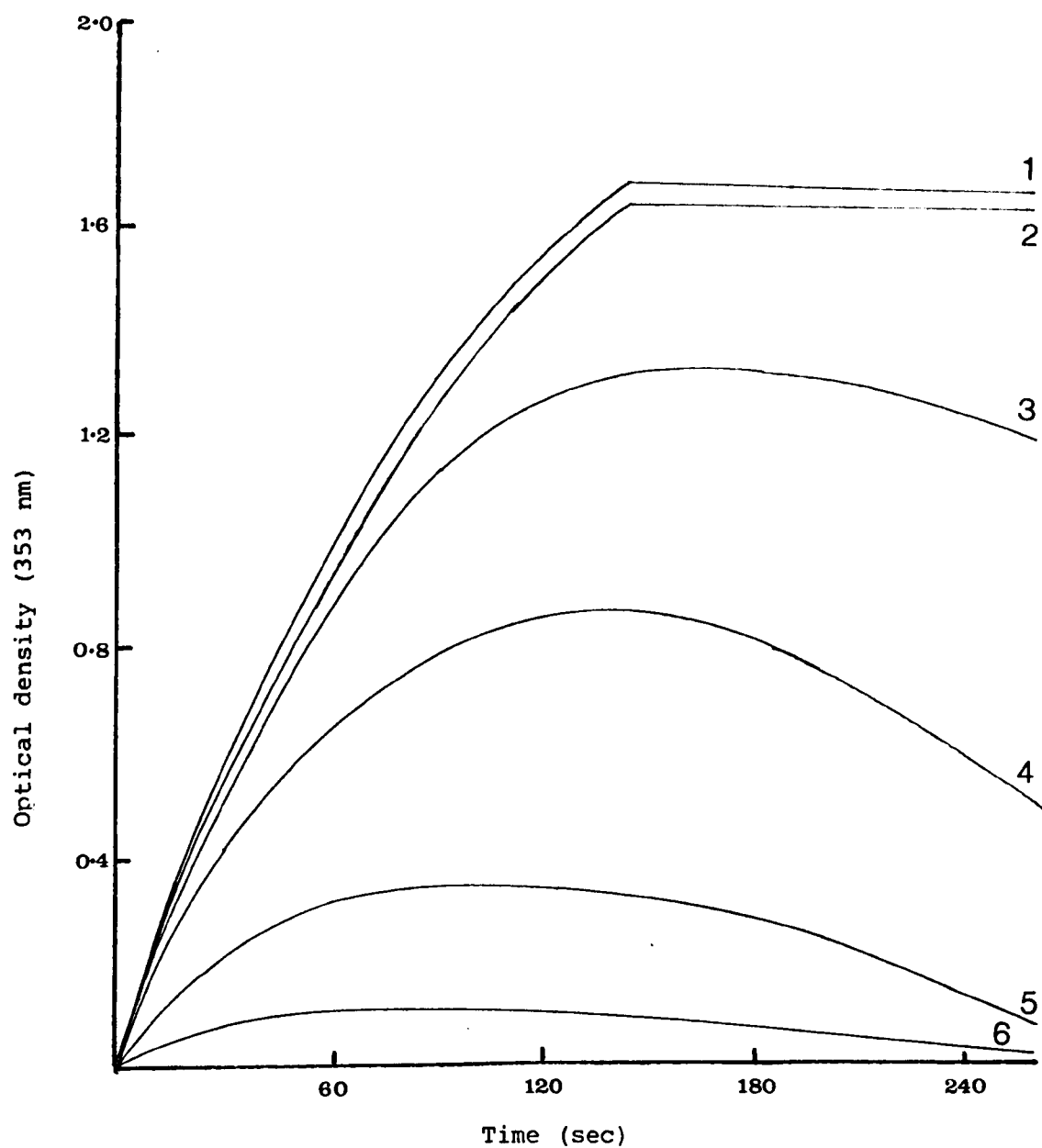


Figure 5.2 Influence of SCN on the rate of HRPO catalysed oxidation of I by H_2O_2 . (1) no NH_4SCN , (2) 0.5 ml of 0.02 mM NH_4SCN , (3) 0.5 ml of 0.4 mM NH_4SCN , (4) 0.5 ml of 1.32 mM NH_4SCN , (5) 0.5 ml of 5mM NH_4SCN and (6) 0.5 ml of 10 mM NH_4SCN .

observed. At 5 and 10 mM maximum I_3 formation was observed after 80 and 60 sec respectively followed by a decline. With the other levels, maximum formation was observed at the same time as when I^- alone was employed (145 sec). This was followed by a decline in the level of I_3 absorbance which was most rapid for the highest of these concentrations.

That the effects produced by NH_4SCN addition are due to the SCN moiety can be seen from figure 5.3. $NaSCN$ and NH_4SCN (5mM) caused considerable inhibition of I_3 formation whereas $(NH_4)_2SO_4$ and Na_2SO_4 had no effect.

5.A.2.3 Thiocyanate content of the test plants

Results for the analyses for the potential SCN concentrations in the four species of plants are given in table 5.1. These revealed

Table 5.1 The thiocyanate content of bean, pea, kale and cabbage

Plant species	thiocyanate content
bean	200
pea	380
kale	2160
cabbage	1910

Values are in μg per 100g of fresh tissue and are means of two replicates

that the potential content in cabbage and kale are approximately a factor of ten greater than in bean. The concentration in pea was approximately twice that in bean.

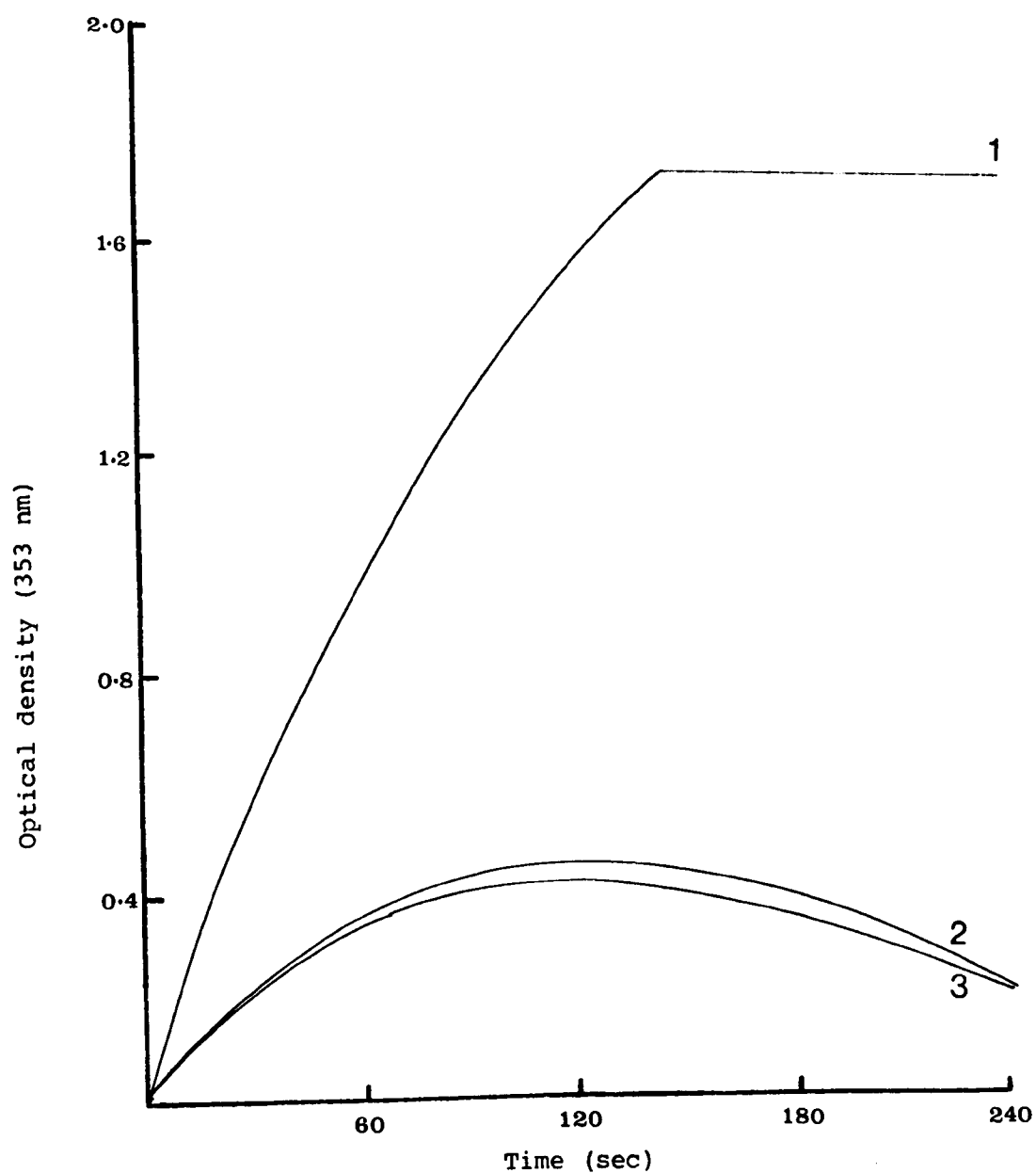


Figure 5.3 Influence of NaSCN, NH_4SCN and $(\text{NH}_4)_2\text{SO}_4$ on HRPO catalysed oxidation by H_2O_2 . (1) I alone and I + Na_2SO_4 or $(\text{NH}_4)_2\text{SO}_4$, (2) I + NaSCN and (3) I + NH_4SCN . All additives 0.5 ml of 0.5 mM.

5.A.2.4 Selective toxicity of iodide to the various test plants

The results of the assessment of the selective toxicity are given in table 5.2.

Table 5.2 Assessment of the selective toxicity of iodide towards bean, pea, kale and cabbage

Plant species		toxicity rating			
		hours after application			
		24	72	120	168
bean	(1.5)	0	3	4	5
pea	(1.5)	0	1	1	4
kale	(1.5)	0	0	0	0
cabbage	(1.5)	0	0	0	0
bean	(15)	1	4	5	5
pea	(15)	0	3	3	5
kale	(15)	0	0	0	0
cabbage	(15)	0	0	0	0
bean	(75)	2	5	5	5
pea	(75)	1	4	5	5
kale	(75)	0	1	1	1
cabbage	(75)	0	0	0	1
bean	(150)	3	5	5	5
pea	(150)	2	5	5	5
kale	(150)	0	1	1	2
cabbage	(150)	0	0	1	1

Toxicity assessed as 0 = no effect through to 5 = complete dessication of the plant. Application rates of NaI in mg/pot are in parentheses.

These show that bean was most susceptible, an application of 1.5 mg I per pot was sufficient to completely dessicate the plant within 120h. Pea was slightly more resistant although complete dessication was observed after 168h at an application rate of 1.5 mg per pot. At the two lowest levels, both kale and cabbage were totally unaffected while at the two higher levels, although some effect was observed, these plants were subsequently found to recover.

5.A.3 Discussion

From the experimental evidence here it can be seen that SCN is capable of inhibiting the oxidation of I by a plant peroxidase system and thus may be a factor in determining the ability of a plant to convert applied I to toxic I_2 and so play a role, at least in part, in the selective action as proposed earlier (5.1). The trends in increasing resistance of the test plants to I appear to be closely related to increasing concentrations of SCN in the plants.

The levels of SCN found to be present in kale and cabbage was considerably reduced compared with the concentrations found by Johnston and Jones (1966) and Paxman and Hill (1974a). However, the plants used in their studies were field grown and generally more mature, while the ones used in this study were young, glasshouse grown plants. Other workers have indicated that age, variety and environmental factors can exert considerable influence on SCN concentrations (Josefsson and Appelqvist, 1968; Neil and Bible, 1972; Bible et al., 1980).

Several aspects arose out of this study which it was felt merited further investigation:

- (1) The possibility of introducing resistance to I in previously susceptible plants is explored in 5.B.
- (2) When higher levels of SCN were employed in the enzyme study, the slowly formed I_3 was found to rapidly disappear again (figure 5.2). I_3 disappearance could be due to reaction with the peroxidase protein (Ramachandran, 1956), however, when I alone was incubated the I_3 was found to be stable over at least 15 min (little drop in absorbance reading). It seems unlikely that SCN could have enhanced this in some way. It was decided to look more fully into the mechanism of SCN inhibition of the I oxidation - see 5.C.
- (3) SCN may not be the only substance released on cell damage which may affect the levels of I_2 able to exert its toxic effect. Other goitrogens or natural plant constituents may cause inhibitory effects and thus contribute to selectivity. This aspect is investigated in 5.D.

5.B Investigation into conferring resistance to iodide in dwarf bean by thiocyanate application

5.B.1 Introduction

Since SCN inhibits I oxidation to toxic I_2 by peroxidase and a good relationship between increasing SCN content and resistance to I was observed, it was decided to investigate the possibility of conferring resistance in dwarf bean by either the pre-treatment or simultaneous

addition of SCN. Dwarf beans were chosen because of their high susceptibility to I and their low potential SCN content - see 5.A.

5.B.2 Experimental

Plants were sown into seed trays containing vermiculite and maintained in a growth room at $30 \pm 3^\circ\text{C}$ under artificial lighting with a 16h day length. Plants were selected on a basis of uniformity of size and lack of damage. They were transplanted into 10 cm pots (no drainage holes) containing vermiculite when the primary leaves were fully expanded (11 days). Preliminary experiments using a range of I and SCN levels showed that 2 mg of NaI per pot, applied in the watering solution, gave a suitable toxic response after 5 days. The maximum that could be applied before NH_4SCN produced toxic symptoms was 30mg/pot. During these experiments it was first noticed that at lower levels of I and SCN considerable defoliation occurs after 5-8 days, whereas at higher levels dessication without leaf fall takes place. The levels required to produce these responses were about five times lower for I than for SCN. These findings are investigated and discussed in chapter 6.

On commencing the study (day 0), twenty four pots were taken and half were treated with 30mg/pot of NH_4SCN . NaI (2mg/pot) was added to water or SCN treated plants (3 per regime) after 0, 1 and 2 days. The pots were held at $25 \pm 0.75^\circ\text{C}$ with a 16h day length for the duration of the experiment. Assessment was made after 3, 5 and 7 days using an arbitrary 0-5 scale (0 = healthy, 5 = severe dessication, defoliation or dead). Control plants showed no effect over 7 days.

5.B.3 Results and Discussion

Problems with assessment and interpretation occurred due to the forementioned defoliation of the primary leaves in many of the treated plants, in one case by day 5, and the start of toxic effects on the plants by the SCN itself.

The results of the study indicate that I toxicity to dwarf bean can be temporarily decreased by simultaneous or pre-addition of SCN to a limited extent. When I and SCN were added simultaneously at day 0 protection from I is given from day 3 to day 7, more so than when SCN is added one day prior to I application. If SCN is added 2 days prior to I then no overall protection is afforded (figure 5.4).

The decreased ability of SCN to protect when added prior to I may be due to the increased effect of the SCN itself. The plant may rapidly convert SCN to bound or oxidised forms which may result in SCN loss before it has a chance to affect I oxidation or the dilution of the available SCN to a level not sufficient to be effective.

The temporary protection may have been due to SCN effect on I uptake and translocation by the roots, thus a similar experiment was designed using foliar sprayed SCN and root applied I. The results obtained were similar to those found when both ions were root applied. Experiments using various levels of root applied SCN in attempts to improve the protection effect of SCN proved fruitless, either not enough SCN for protection or too much and so be toxic itself was found to occur.

Thus the adverse effects of SCN itself appear to limit the practicality of conferring resistance to I toxicity in dwarf bean. Time did not permit further more thorough investigation using other plant

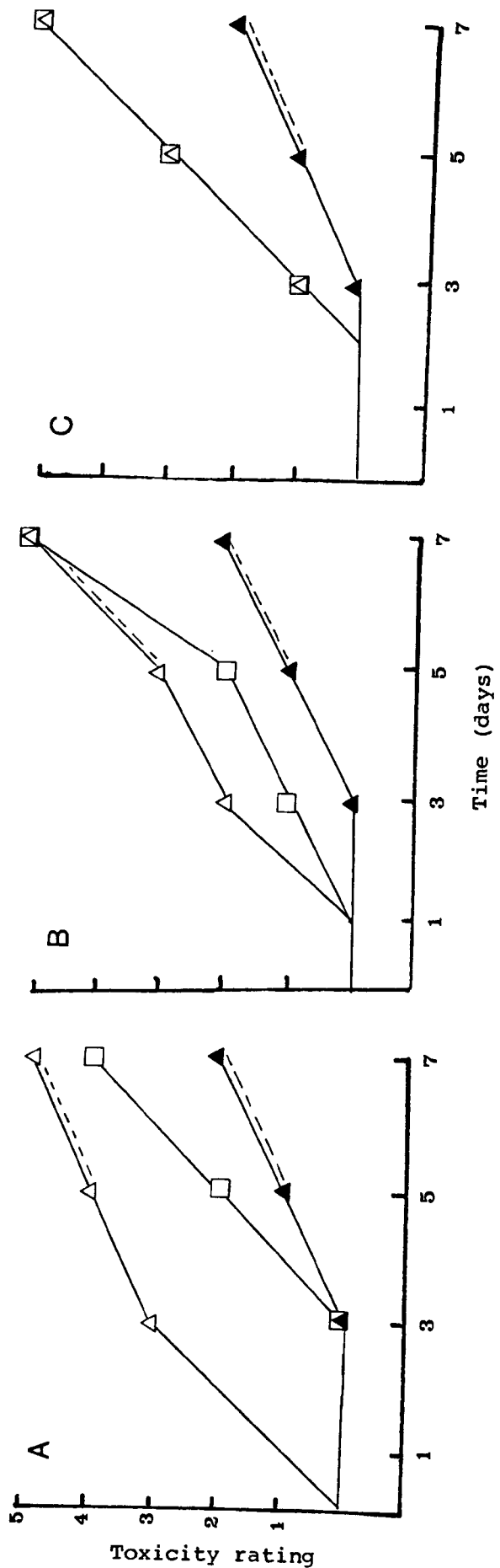


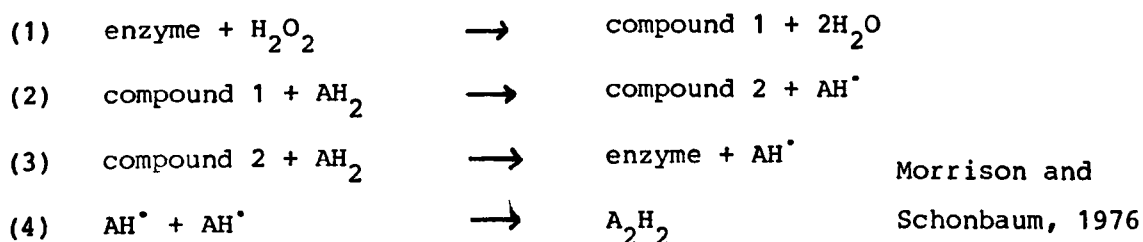
Figure 5.4 Influence of NH_4SCN additions on I toxicity to dwarf bean. Any NH_4SCN treatment (30 mg per pot) carried out on day 0. I treatments (2 mg per pot) carried out on A - day 0, B - day 1 and C - day 2. \triangle NaI alone, \square NaI + NH_4SCN and \blacktriangle NH_4SCN alone.

species and environmental conditions, although in the wake of the following chapter, improved results could be achieved if similar experiments were carried out in diffuse light or darkness. Mr. D. Al-Jaff is continuing on aspects of this work in this laboratory.

5.C Investigation into the mechanism of action of thiocyanate inhibition of HRPO catalysed iodide oxidation

5.C.1 Introduction

The mode of action of peroxidase is complex and is yet not fully understood. A simplified probable scheme is given below:



It is probable that free radical reactions are involved (Morrison and Schonbaum, 1976; Yamazaki, 1976). As discussed previously (chapter 4), SCN is an efficient free radical scavenger (Adams et al., 1972) and this may be an important factor in its inhibitory effect. Presumably any scavenging activity of I itself will equilibrate out when incubated alone.

In mammalian thyroids, SCN has been shown to inhibit the oxidation of I. It has been suggested that this was through its ability to be oxidised by thyroid peroxidases, thus competing with I for oxidising equivalents (Maloof and Soodak, 1964; Aune et al., 1977). SCN has been shown to be oxidised by several non-phytoperoxidases (Morrison and

Schonbaum, 1976), however, neither Chung and Wood (1970) nor Sorbo and Ljunggren (1958) could demonstrate similar properties with HRPO. SCN is considered by chemists to be a pseudohalide, possessing similar electronic structure and properties to the halides (Hughes, 1975). Thus it may inhibit the oxidation of I by binding to the peroxidase at the active site without being oxidised itself and so acts as a competitive inhibitor. Morita (1958), using various phytoperoxidases (not including HRPO), has shown that SCN inhibited peroxidase catalysed oxidation of guaiacol in an irreversible manner by causing splitting of the hematin portion of the enzyme. Randall (1946) showed that the apparent anti-peroxidase activity of certain thiol compounds were due to their reducing power on the coloured complexes formed, rather than a true inhibitory effect on peroxidase.

5.C.2 Investigation into possible thiocyanate oxidation by HRPO

The possibility that SCN may compete for oxidising equivalents with I by being itself oxidised by HRPO was investigated. The presence of sulphate would indicate that SCN oxidation had taken place in the presence of I (Oran and Reuter, 1966). It was proposed to add BaCl_2 to incubation mixtures and determine SO_4 by indirect measurement of unprecipitated Ba by atomic absorption spectrophotometry (Dunk et al., 1969), however, interferences due to the precipitation of the Ba with both the buffer and the I_3 oxidation product ruled out this prospect.

SCN was incubated as per 5.A.1.2.2 in the absence of I. No decrease in absorbance at 235 nm was observed after 1h, indicating no disappearance of SCN under these conditions. This method could not be

applied when I was present since the strong uv absorbance of I and I_3 masked the absorbance maximum of SCN.

The addition of $Fe(NO_3)_3$ (1 ml of 0.4M in 1N HNO_3) was made to similar incubation mixtures and the brick red colour of the iron-SCN complex read at 430 nm. In the absence of I no decrease in SCN levels were detected. $Fe(NO_3)_3$ reacted in the presence of I or I_3 giving a large non-specific uv absorbance which affected the readings. However, if the optical density was read almost immediately after $Fe(NO_3)_3$ addition then zero background levels could be obtained. SCN levels before and after incubation under similar conditions employed in 5.A.1.2.1 are shown in table 5.3.

Table 5.3 Thiocyanate levels before and after incubation with an iodide/HRPO/hydrogen peroxide system

Treatment	Thiocyanate concentration
time 0 ^a	4.8
time 4 min ^a	2.5
time 0 min ^b	9.5
time 4 min ^b	7.0

^a 0.5 ml of 0.5 mM NH_4SCN employed

^b 0.5 ml of 1 mM NH_4SCN employed.

Thiocyanate concentrations are in $\mu g/ml$ of the final reaction mixture

Considerable decrease in SCN content occurred after incubation with I, indicating oxidation of SCN under the reaction conditions.

5.C.3 Investigation into possible thiocyanate effects on HRPO itself

The possibility that an irreversible effect on HRPO due to SCN was considered. The purity of the enzyme preparation available at the time of the study was not suitable for the spectral investigation techniques as used by Morita (1958). However, when SCN was pre-incubated with the enzyme for 1h at 37°C prior to I and H₂O₂ addition (as 5.A.1.2.2), no decrease in activity over standard 10 min equilibrium conditions were observed.

5.C.4 Kinetic study of thiocyanate inhibition of iodide oxidation by HRPO

A conventional kinetic study was applied in an attempt to ascertain if any of the characteristic effects of reversible inhibitors (Morris, 1974) on a double reciprocal plot could be distinguished.

The reaction conditions employed were similar to those in 5.A.1.2.2 except that 0.1 ml of 0.05 mg/ml of HRPO was used and the I levels were changed in order to give a range suitable for plotting. The rates of reaction (V_o) were determined over the first two min of the reaction in terms of abs. units/min at 353 nm. The Lineweaver-Burk (1934) plot of $1/V_o$ versus the reciprocal of the substrate concentration $1/[S]$ (figure 5.5) is not very helpful in that the inhibitor produced a non-linear plot. This makes interpretation impossible but it does tend to suggest that inhibition of a complex nature is taking place. It could be said that competitive inhibition is taking place in the sense that the inhibition was overcome at high substrate concentrations.

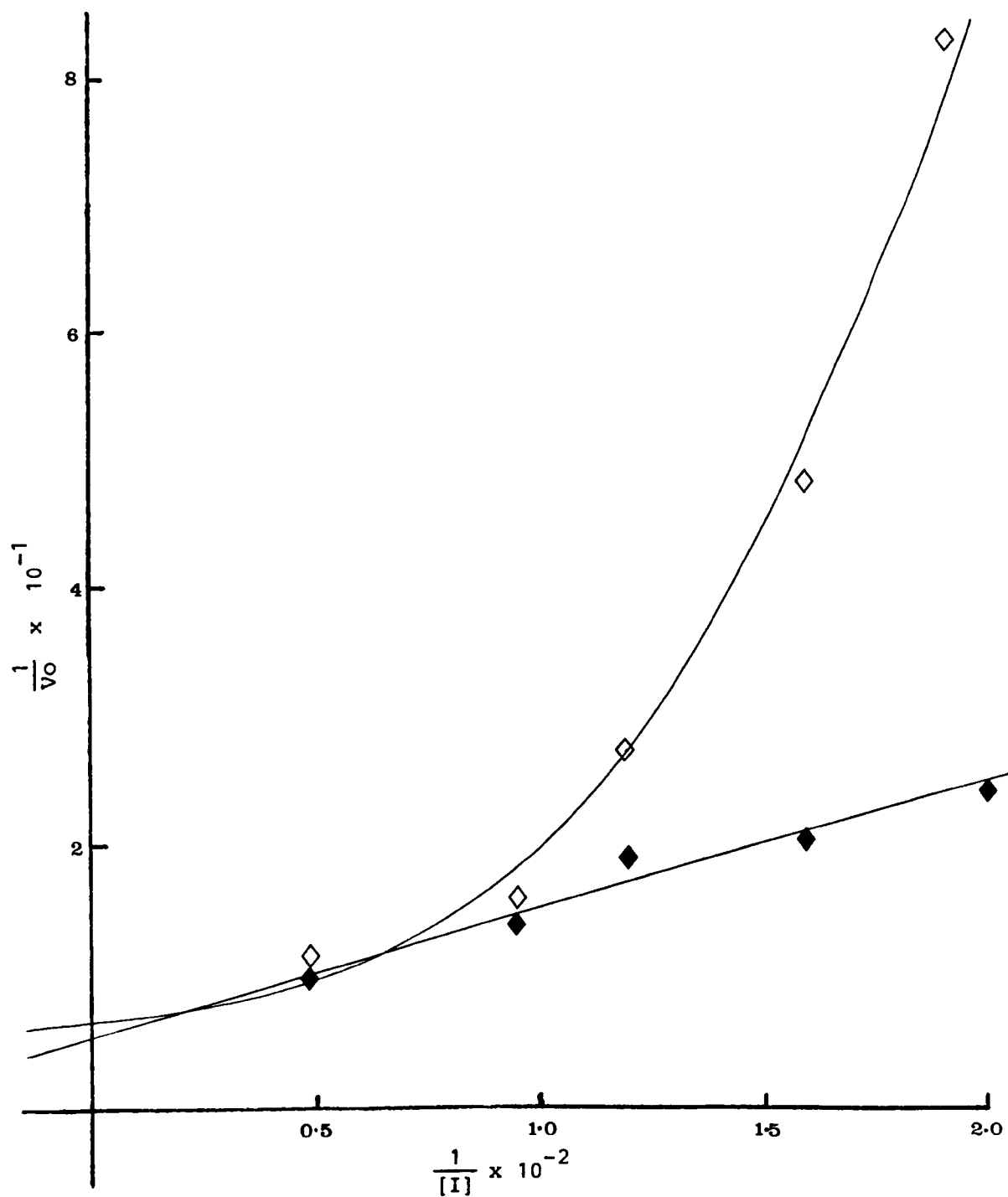


Figure 5.5 Kinetic study on the SCN inhibition of the HRPO catalysed oxidation of I by H_2O_2 . I levels: final concentration of $0.5\text{--}2.11 \times 10^{-2} \text{ M}$. SCN concentration $3 \times 10^{-3} \text{ M}$. \blacklozenge denotes I alone, \diamond I + SCN, V_o = maximum velocity in abs. units at 353 nm/min.

5.C.5 Investigation into triiodide ion disappearance in the presence of thiocyanate

A study was made of the decrease in I_3 levels observed when the higher levels of SCN were present, the possibility existing that the effect may be due to reducing power towards the reaction product, as found by Randall (1946) for thiol containing compounds.

The reaction conditions described above (see 5.A.2.1), whereby I was completely oxidised and H_2O_2 was in slight excess, were employed. The reaction was allowed to go to completion (no I remaining) before 0.1 ml of 5 mM NH_4SCN was added. The course of the reaction is described in figure 5.6. On addition of SCN the I_3 level was seen to fall, at first slowly, then more rapidly, before stabilising at a low level (0.25 abs. units). When an addition of H_2O_2 (100 μ l of 10 mM) was made the I_3 colour again appeared, the rate and extent of formation being slightly less than originally. If followed over a longer period of time, one was able to continue to add in sequence SCN, H_2O_2 , SCN, etc., in appropriate amounts, with the I_3 levels falling on SCN addition and rising again with H_2O_2 . Thus, despite I being originally all oxidised, if SCN was first added and allowed to react, then further H_2O_2 addition resulted in renewed oxidative formation of I_3 .

5.C.6 Discussion

The lack of HRPO catalysed oxidation of SCN in the absence of I (5.C.2) is in keeping with the findings of Sorbo and Ljunggren (1958) and Chung and Wood (1970). The results here suggest that SCN is oxidised only in the presence of I. The inability of HRPO to oxidise SCN is

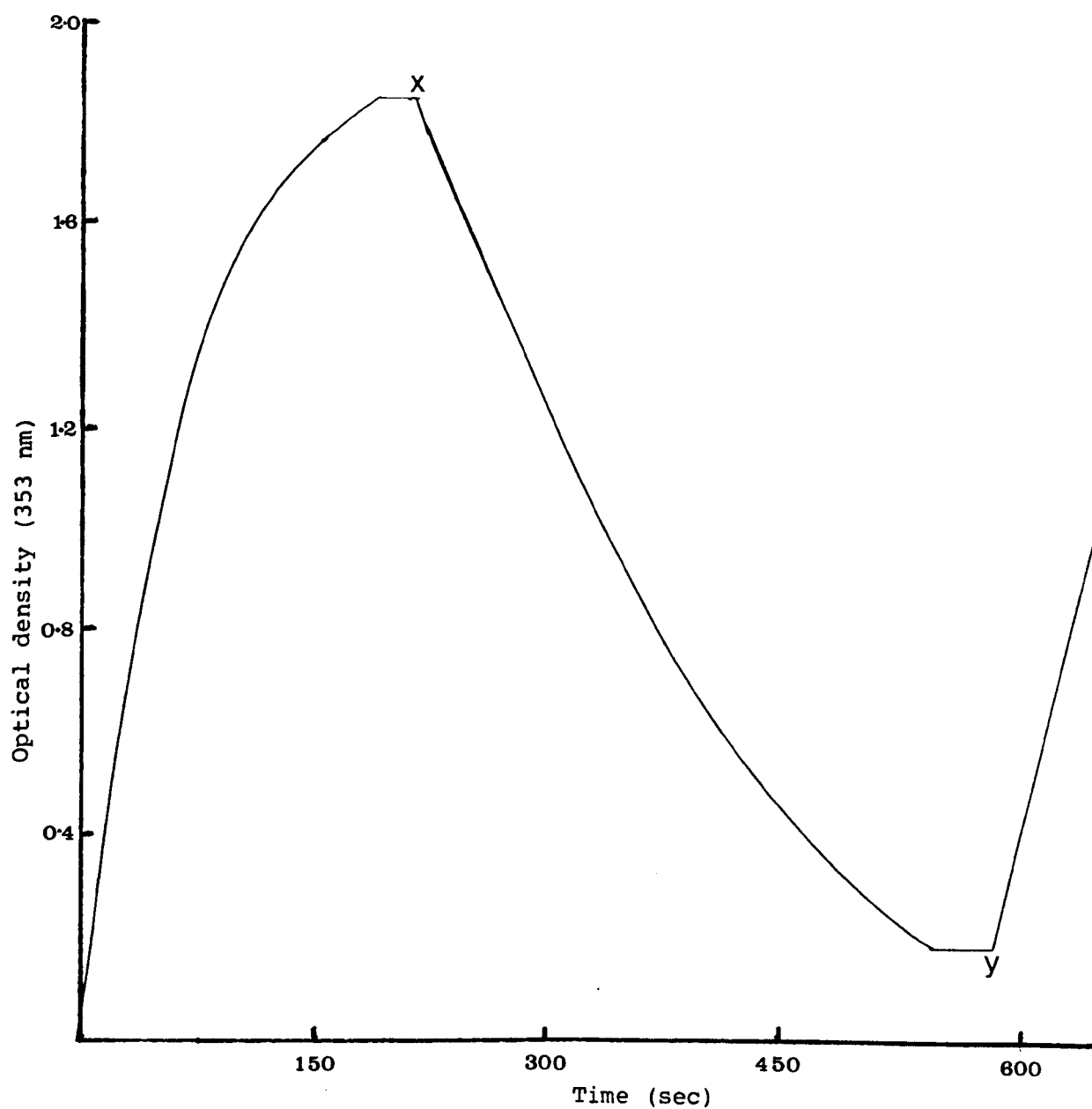
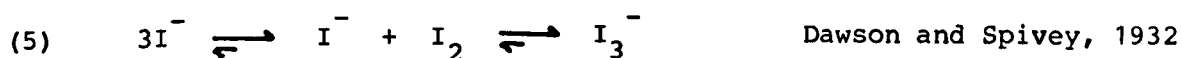


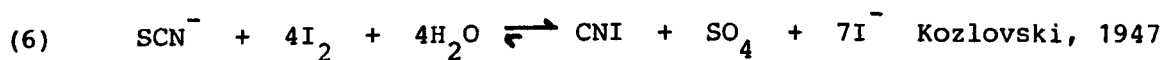
Figure 5.6 Influence of addition of SCN after completion of HRPO catalysed oxidation of I by H_2O_2 . Reaction conditions employed whereby all I oxidised before addition of NH_4SCN (0.1 ml 5 mM) at X. Further addition of H_2O_2 (100 μl 10 mM) made at Y.

almost certain to be due to the particular peroxidase-peroxide initial complex (compound 1 - the oxidant of the halide ion - see eqn. 2 in 5.C.1) not possessing the redox potential necessary for SCN oxidation. The oxidation potential though is sufficient for I oxidation (Morrison and Shonbaum, 1976). It seems unlikely that the oxidation of I would sufficiently alter the potential of compound 1 to allow enzymic oxidation to take place. The free radical scavenging properties of SCN (Adams *et al.*, 1972) may enable it to react with radicals involved in the peroxidase mechanism of action, if indeed they are involved. This would involve oxidation of SCN. I is also an efficient free radical scavenger, more so than SCN (Bellus, 1978), thus I itself would be working here. The degree to which such preferential oxidation of SCN would take place would thus be difficult to establish.

The formation of I_3 is a result of the enzymic oxidation product I_2 reacting with unoxidised I thus:



A study of the literature showed no reported chemical reaction of I_3 with SCN, however, SCN will react at neutral pH's with I_2 :



The reaction proceeds via a I_2 -SCN complex and the equilibrium is towards the reduction of I_2 (Lewis and Skoog, 1962). Taken in conjunction with eqn. 5 this could result in a change of equilibrium away from I_3 formation resulting in I regeneration. The results of 5.C.5 strongly suggest that such is taking place in the presence of SCN. This would also account for the observed SCN disappearance (5.C.2).

Thus, it would appear that under the conditions employed in the original study (5.A.1.2.2), the observed inhibition by SCN could be due to not true inhibition of the enzyme itself but to the chemical reducing power of the SCN on the oxidation products. Competition for oxidising equivalents (compound 1) seems unlikely, although scavenging of OH^\bullet radicals involving SCN oxidation may occur. Competitive inhibition or irreversible effects on the enzyme cannot be ruled out at this stage. The Lineweaver-Burk Plot inferred some non-competitive inhibition taking place (5.C.4) but the non-linearity of $1/V_o$ with respect to $1/[S]$ in the presence of SCN confused this. The non-linearity is probably due to the non-enzymic reduction reactions resulting in apparently less I_3 being formed than only true enzyme inhibition would have accounted for. In a brief investigation, the effect of SCN on the oxidation of PABA by the $\text{HRPO}/\text{H}_2\text{O}_2$ system was carried out - see also 5.D.3.4. SCN was found not to reduce the PABA-red reaction product. Competitive oxidation by SCN with respect to PABA was observed. It could be concluded that; a) reversible binding of SCN takes place on the HRPO molecule; b) this takes place sufficiently near the active site of PABA for hinderance to take place and thus c) SCN binding affecting I oxidation may be possible provided the binding site is close to the PABA/SCN site. As postulated above (5.C.1), I and SCN may share the same binding site.

5.D The possible role of substances other than thiocyanate in the selective herbicidal action of iodide

5.D.1 Introduction

Production of compounds or ions other than SCN on cell damage may affect the formation of toxic I_2 from I. Langer (1966) has shown that the goitrogenicity of cabbage and other crucifers to mammals is due not just to the SCN produced, but to the combined action of many of the hydrolysis products of the particular glucosinolate precursors present. These products can include SCN, isothiocyanates, goitrin (vinylloxazolidine-thiones) and others, all of which are active goitrogens in their own right. Glucosinolates are abundant in crucifers, over fifty different structures being isolated. Although only one or two are usually present in relatively large amounts, as many as six of these compounds have been found in a given species (Ettlinger and Kjaer, 1968).

Organic nitriles are also products of glucosinolate hydrolysis, though non-goitrogenic (Van Etten et al., 1969). The cyanide ultimately produced from these nitriles or from cyanogenic glycosides have also been shown to be goitrogenic due to their conversion to SCN (Van der Velden et al., 1973). CN itself is one of a group of anions reported to inhibit plant peroxidases (Morita, 1954).

Goitrogenic effects on rats have also been demonstrated with the commercially used dithiocarbamate fungicides zineb (zinc ethylene-1,2-bis-dithiocarbamate) and nabam (disodium ethylene bis-dithiocarbamate) (Smith et al., 1953). The anti-thyroid agent thiourea has been implicated in inhibition of peroxidase catalysed reactions, both through its ability to be co-oxidised in the enzyme system (Morris and Hager, 1966) and its reductive properties (Randall, 1946) similar to those demonstrated for SCN above (5.C).

The aim of this study was to briefly investigate several goitrogenic substances, fungicides, anions and reducing agents in a manner similar to the SCN studies above, i.e., on oxidation of I by the HRPO/H₂O₂ system and in plant growth studies alongside I.

5.D.2 Experimental

5.D.2.1 Materials

Allyl isothiocyanate and thiourea were purchased from Koch Light Laboratories. Zineb was purchased from Pan Britannica Industries as a 75% wettable powder. All other reagents were as above (5.A.1.1).

5.D.2.2 Methods

5.D.2.2.1 Influence of various goitrogens on the HRPO catalysed oxidation of iodide by hydrogen peroxide

The oxidation of I was carried out as per 5.A.1.2.1 with, in place of water, 0.5 ml of thiourea (0.25 and 5 mM), zineb (0.25, 1 and 5 mM) and allyl isothiocyanate - a major mustard oil component (5 mM). Zineb was prepared in 1% ethanol to aid solubility. Herrlinger (1948) has shown that alcohol, up to a final concentration of 1%, has no effect on the activity of plant peroxidases. Allyl isothiocyanate was dissolved in ethanol and 50 µl added to the reaction mixtures along with 0.5 ml of water - this approximates to addition of 0.5 ml of 5 mM in terms of the other additives. Appropriate ethanol blanks were incorporated. In several cases addition of goitrogen was made after incubation under I limiting conditions (see 5.A.2.1) as used for SCN above (5.C.5).

5.D.2.2.2 Influence of various anions on the HRPO catalysed oxidation of iodide by hydrogen peroxide

The oxidation of I was carried out as per 5.A.1.2.1 with 0.5 ml of 5 mM fluoride, chloride, bromide and azide added. Reactions were also carried out with 0.5 ml of 0.25, 0.5 and 5 mM cyanide. All additives were as the sodium salts. CN and N_3 were also added after incubation under I limiting conditions (5.A.2.1).

5.D.2.2.3 Influence of various reducing agents on the HRPO catalysed oxidation of iodide by hydrogen peroxide

I oxidations were carried out as per 5.A.1.2.1 with as additive 0.5 ml of ascorbic acid (0.25 and 5 mM) cysteine and dithionite (1 and 5 mM). Addition of reducing agents were also made after incubation under I limiting conditions (5.A.2.1).

5.D.2.2.4 Influence of various additives on the HRPO catalysed oxidation of PABA by hydrogen peroxide

Incubations were carried out as for I (5.A.1.2.1) with the exception that 0.5 ml of 10 mM PABA was employed and the rate of formation of PABA-red azo dye at 474 nm was followed. Additives employed were 0.5 ml of 5 mM NH_4SCN , ascorbic acid, cysteine, thiourea, NaCN and NaN_3 . Ascorbic acid (0.25 mM) and cysteine (0.5 mM) were also tested.

5.D.2.2.5 Influence of various additives on the toxicity of I to dwarf bean

Dwarf bean plants were reared and potted out as per 5.B.2. Root applications of I (2mg/pot) were made with the simultaneous addition

of ascorbic acid (70mg/pot), thiourea (30mg/pot), zineb (40mg formulation/pot), and NaCN (25mg/pot). These levels, except zineb, are approximately equimolar to the NH_4SCN at 30mg/pot employed in 5.B.2. Three plants per treatment were employed and appropriate controls used. The plants were grown as per 5.B.2 and similar arbitrary assessment made after 4 and 7 days. Control plants (no treatment) showed no effect after 7 days.

The effect of several of the chemicals were further assessed using adjusted levels of additive.

5.D.3 Results

5.D.3.1 Influence of various goitrogens on the HRPO catalysed oxidation of iodide by hydrogen peroxide

The effect of these goiter inducing substances can be seen in figure 5.7. At the 5 mM additive level zineb and thiourea completely inhibited the oxidations. Allyl isothiocyanate (5 mM) had no effect. The addition of zineb at 0.25 mM produced a delay in the appearance of I_3 colour of 25 sec, thereafter the rate of reaction was similar to that where no additive was present. The final absorbance level, after a similar time (148 sec) was lower than for I alone, seemingly the result of the initial lag period. At 1 mM zineb the lag time was proportionally longer than for the 0.25 mM level (96 sec), thereafter the rate of reaction was slower than that with no additive and the reaction ended after 232 sec.*

Addition of 50 μl of 5 mM thiourea or zineb after completion of I oxidation can be seen in figure 5.8. An almost instantaneous drop

* After a time lag of 128s, the rate of reaction in the presence of 0.25mM was slower than when no additive was present. The overall reaction time was longer (236s).

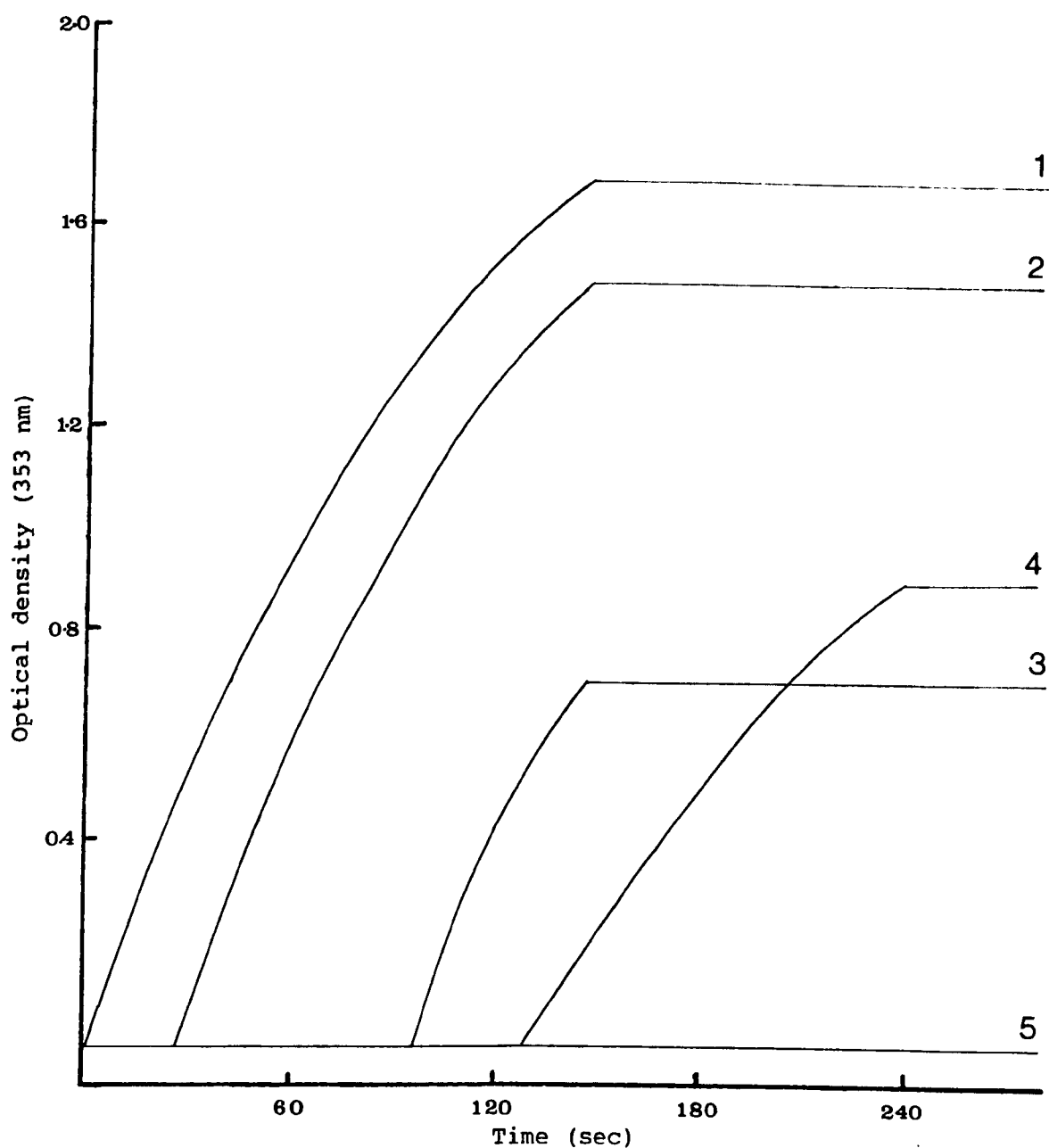


Figure 5.7 Influence of various goitrogenic substances on the HRPO catalysed oxidation of I by H_2O_2 . Treatments: (1) I alone and I + allyl isothiocyanate (0.5 ml 5 mM), (2) I + zineb (0.5 ml 0.25 mM), (3) I + zineb (0.5 ml 1 mM), (4) I + thiourea (0.5 ml 0.25 mM) and (5) I + zineb or thiourea (0.5 ml 5 mM).

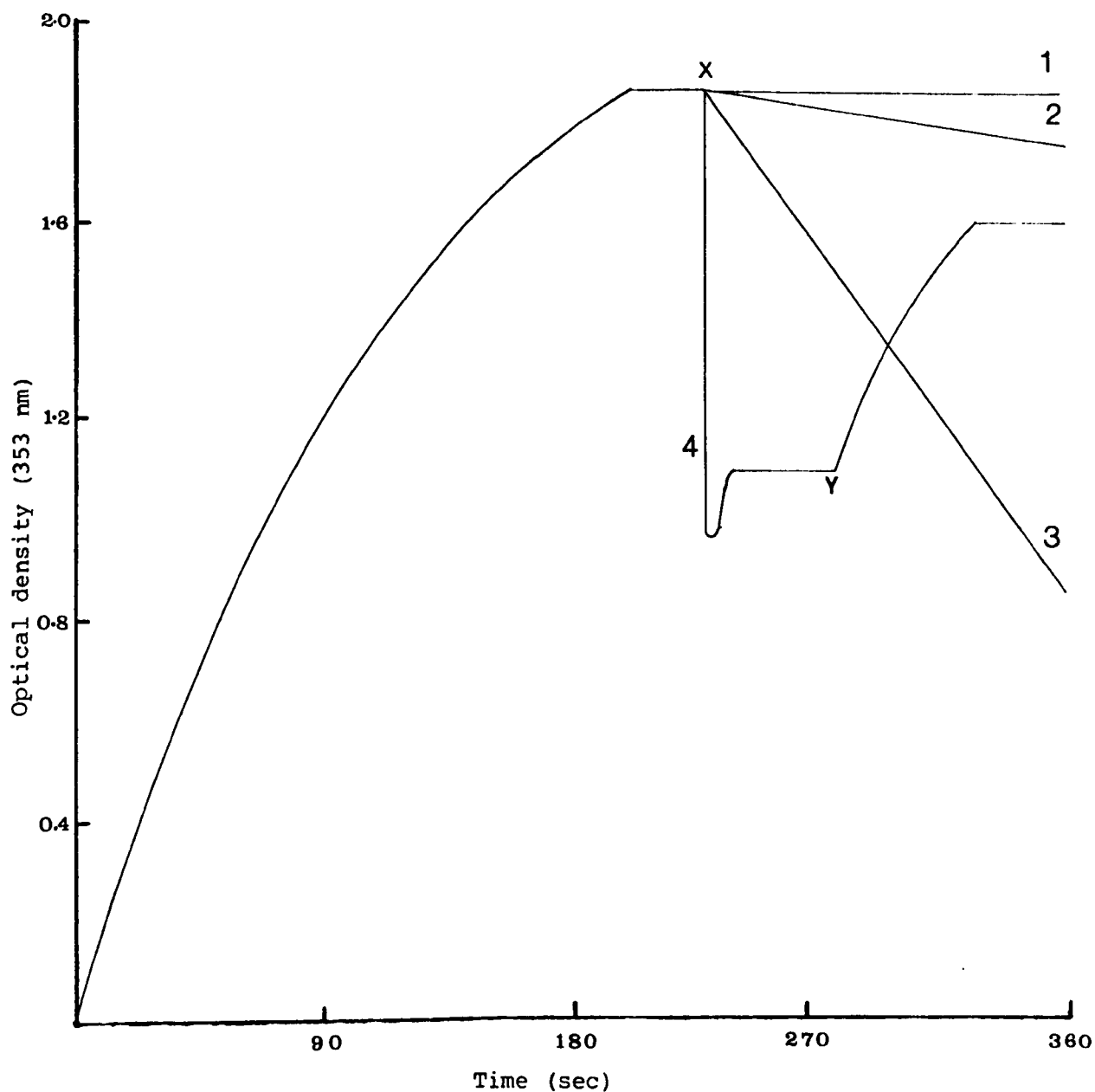


Figure 5.8 Influence of various inhibitors added after completion of the HRPO catalysed oxidation of I by H_2O_2 . Reaction conditions employed whereby all I oxidised before addition at X of (2) $100\mu\text{l}$ of 0.5 mM NaN_3 , (3) $100\mu\text{l}$ $0.5\text{ mM NH}_4\text{SCN}$, (4) $50\mu\text{l}$ 5 mM NaCN , ascorbic acid, zineb, thiourea or cysteine. (1) no addition. A further $50\mu\text{l}$ of H_2O_2 (10 mM) added to 4 at Y.

in I_3 absorption occurring from 1.85 abs. units to approximately 0.96. This was followed by a slight rise to 1.11 over 16 sec. Further addition of H_2O_2 (50 μ l of 10 mM) resulted in re-formation of I_3 colour.

5.D.3.2 Influence of various anions on the HRPO catalysed oxidation of iodide by hydrogen peroxide

At the 5 mM level of additive, F, Cl and Br had no effect on I_3 formation. CN at 5 mM completely inhibited the reaction. At lower levels of CN (0.25 and 0.5 mM), a time lag before production of I_3 occurred (31 and 58 sec respectively), thereafter the rate of reaction was slightly slower than for I alone, the H_2O_2 expiring after 155 and 182 sec. A different pattern of inhibition was produced by N_3 (5 mM); no initial delay in I_3 formation occurred and the overall rate of reaction was considerably slower than for I alone. The end of the reaction occurred after 260 sec with the final I_3 level similar to that for I alone (figure 5.9).

Addition of 50 μ l of CN (equivalent to 0.5 ml of 5 mM if added initially) after completion of I oxidation gave a similar pattern to that found for zineb and thiourea above (5.D.3.1). NaN_3 addition resulted in only a very slow drop in I_3 colour (figure 5.8).

5.D.3.3 Influence of various reducing agents on the HRPO catalysed oxidation of iodide by hydrogen peroxide

At the 5 mM level all treatments completely inhibited the I_3 formation. Ascorbic acid at 0.25 mM produced an initial delay in reaction of 80 sec, thereafter the rate of oxidation was slower than for I alone. The end of reaction occurred after 255 sec. Cysteine and dithionite at

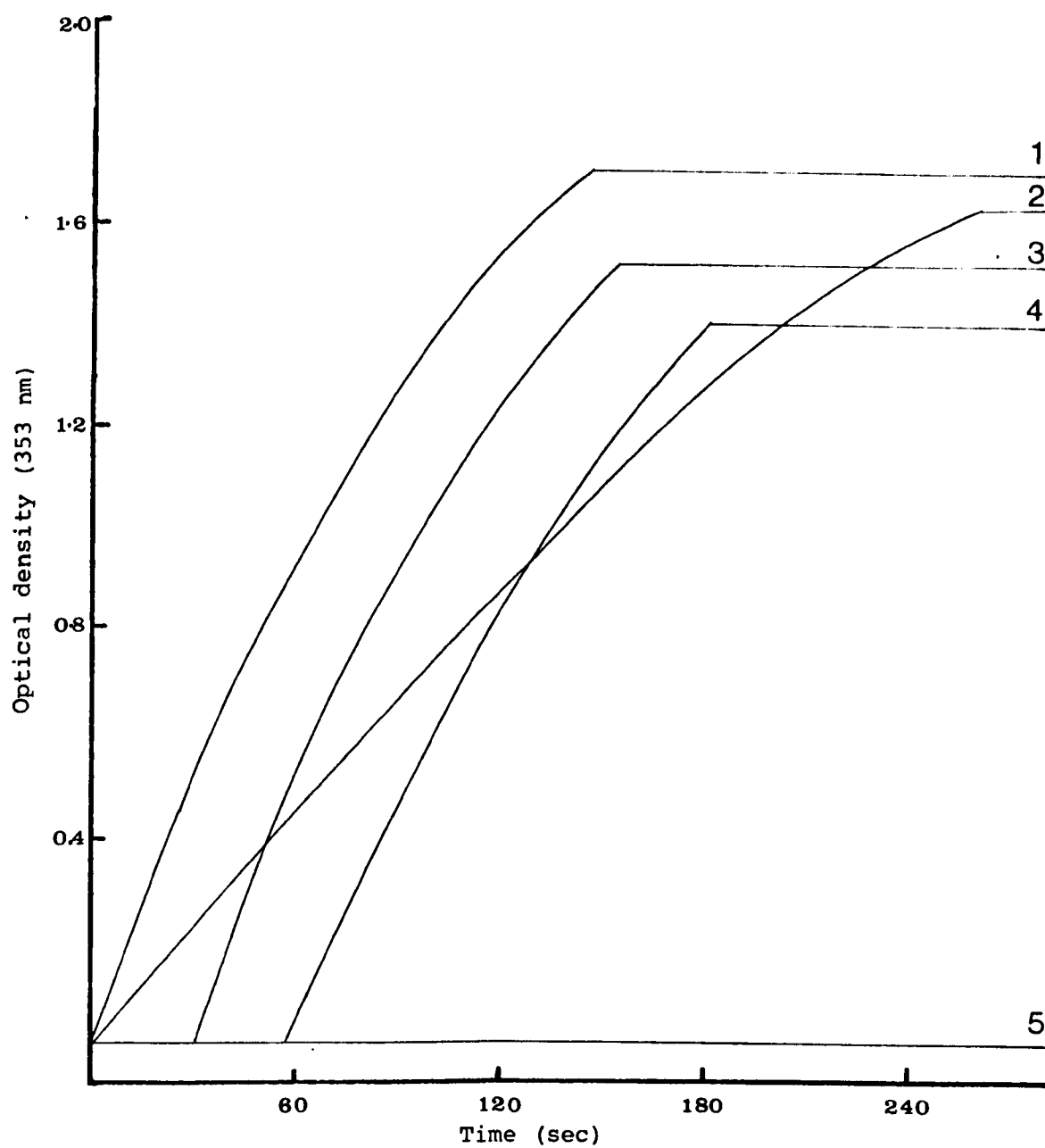


Figure 5.9 Influence of various anions on the HRPO catalysed oxidation of I by H_2O_2 . Treatments: (1) I alone and I + Cl, Br or F (all 0.5 ml of 5 mM), (2) I + N_3 (0.5 ml 5 mM), (3) I + CN (0.5 ml 0.25 mM), (4) I + CN (0.5 ml 0.5 mM) and (5) I + CN (0.5 ml 5 mM).

1 mM both resulted in an initial time lag of 90 sec, the reaction rates and finishing times being as for I alone (figure 5.10).

Addition of 50 μ l of cysteine (5 mM) on completion of I oxidation produced a similar pattern to that found for zineb and thiourea (5.D.3.1) - figure 5.8.

5.D.3.4 Influence of various additives on the HRPO catalysed oxidation of PABA by hydrogen peroxide

The oxidation of PABA, as indicated by formation of PABA-red at 474 nm, ran at 0.12 abs. units/min finishing after 205 sec. With the exception of zineb, all the additives inhibited the oxidation to some extent at the 5 mM level. Ascorbic acid and cysteine completely inhibited the reaction. At lower concentrations, these two reducing agents resulted in time lags followed by reaction rates at 0.12 abs. units/min, ending around the same time as PABA alone; the lower overall extent of reaction presumably due to the initial time lag. The other inhibitors produced no initial time lag. Reaction rates (in abs. units/min) were as follows: in the presence of NH_4SCN 0.10; thiourea 0.06; NaCN 0.04 and NaN_3 0.03. Reaction times and extent were correspondingly longer, although the final SCN level was not significantly lower than when no additive was present (figure 5.11).

5.D.3.5 Influence of various additives on the toxicity of iodide to dwarf bean

As observed earlier (5.B.3) I alone produced some defoliation of primary and trifoliate leaves after 7 days (33% primary leaves and several trifoliates).

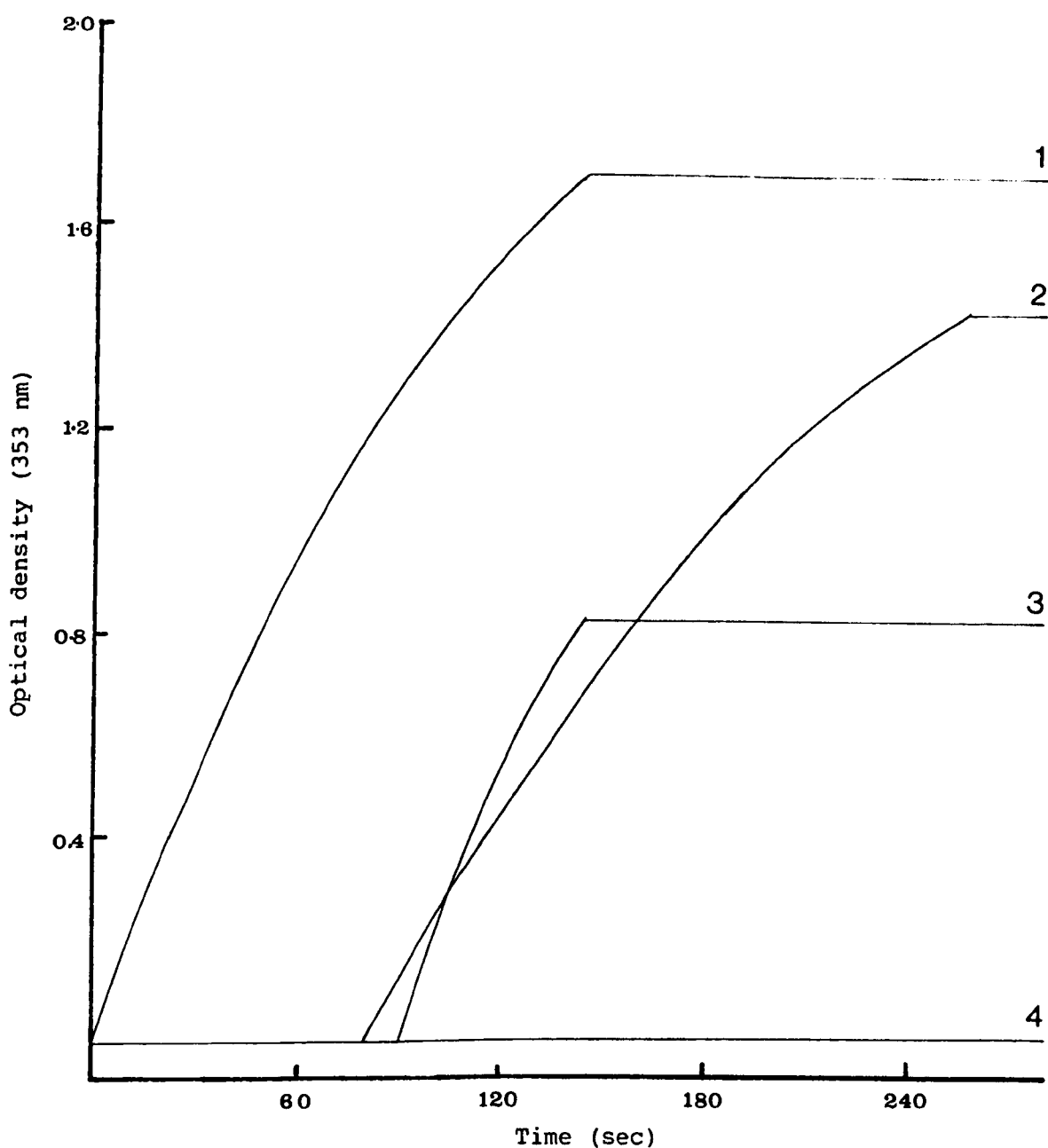


Figure 5.10 Influence of various reducing agents on the HRPO catalysed oxidation of I by H_2O_2 . Treatments: (1) I alone, (2) I + ascorbic acid (0.5 ml 0.25 mM), (3) I + cysteine ^{or dithionite} (0.5 ml of 1 mM) and (4) I + ascorbic acid ^{or dithionite} or cysteine (0.5 ml 5 mM).

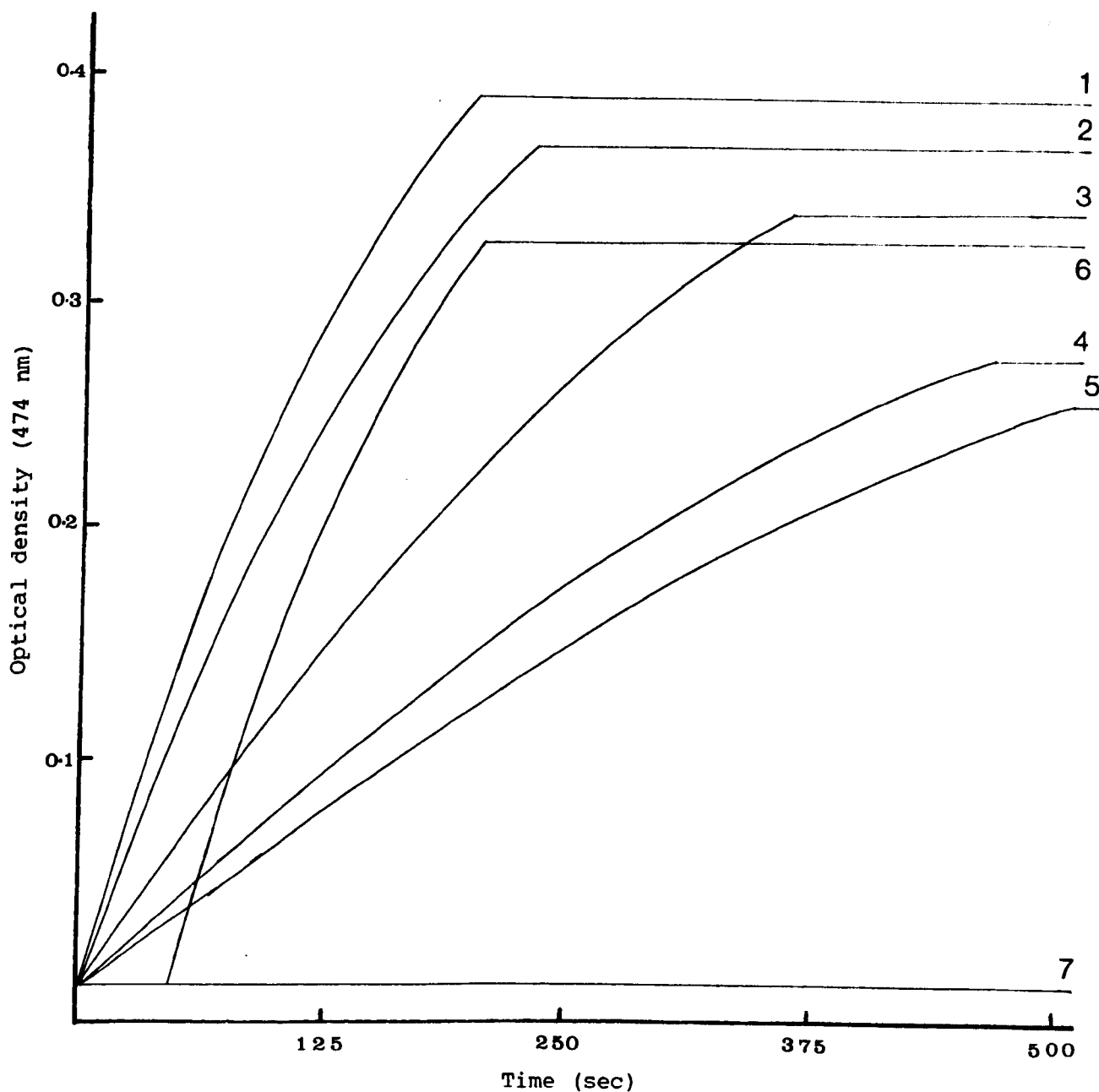


Figure 5.11 Influence of various compounds on the HRPO catalysed oxidation of PABA by H_2O_2 . Treatments: (1) PABA alone and PABA + zineb (0.5 ml 5 mM), (2) PABA + SCN (0.5 ml 5 mM), (3) PABA + thiourea (0.5 ml 5 mM), (4) PABA + CN (0.5 ml 5 mM), (5) PABA + N_3 (0.5 ml 5 mM), (6) PABA + ascorbic acid (0.5 ml 0.25 mM) or cysteine (0.5 ml 0.5 mM) and (7) PABA + ascorbic acid or cysteine (0.5 ml 5 mM).

Ascorbic acid (70 mg/pot) produced a temporary resistance to I toxicity after 4 days. 3 days later no decrease in effectiveness of I was observed (figure 5.12a). Further experiments showed that up to 100 mg/pot of ascorbic acid could be added without any further toxic effect when applied alone. At this level protection against I toxicity was seen to take place after 4 and 7 days (figure 5.13a).

Thiourea alone at 30 mg/pot resulted in considerable defoliation of the bean leaves, 33% of the primary leaves falling after 4 days. After 7 days 66% of primary leaves and several trifoliate leaves had fallen. On addition with I some sign of protection after 4 days was noted but this was difficult to measure and was not borne out after 7 days. The combination with I resulted in almost 100% defoliation (figure 5.12.b). Further studies showed that higher levels of thiourea resulted in increased defoliation or toxicity. At 15 mg/pot thiourea, less effects from the compound alone was noted, however, on addition with I, no difference in I toxicity compared with 30 mg/pot was observed (figure 5.13c). No effect was observed when thiourea was applied at 3 mg/pot. Treatment of I with this level of additive resulted in a marked reduction in I toxicity compared to I alone; only slight defoliation occurred (figure 5.13d).

Zineb at 40 mg/pot produced very little effect on bean after 7 days. Virtually no toxic effects from I were observed when applied with zineb (figure 5.12d). No effect from zineb or zineb plus I was noted when the fungicide was used at a lower level (20 mg/pot) - see figure 5.13b.

CN alone proved very toxic; blackening and dessication occurring after 4 days. These symptoms overshadowed any effect by I (figure 5.12c). CN was toxic at least down to 1 mg/pot and was not considered further for

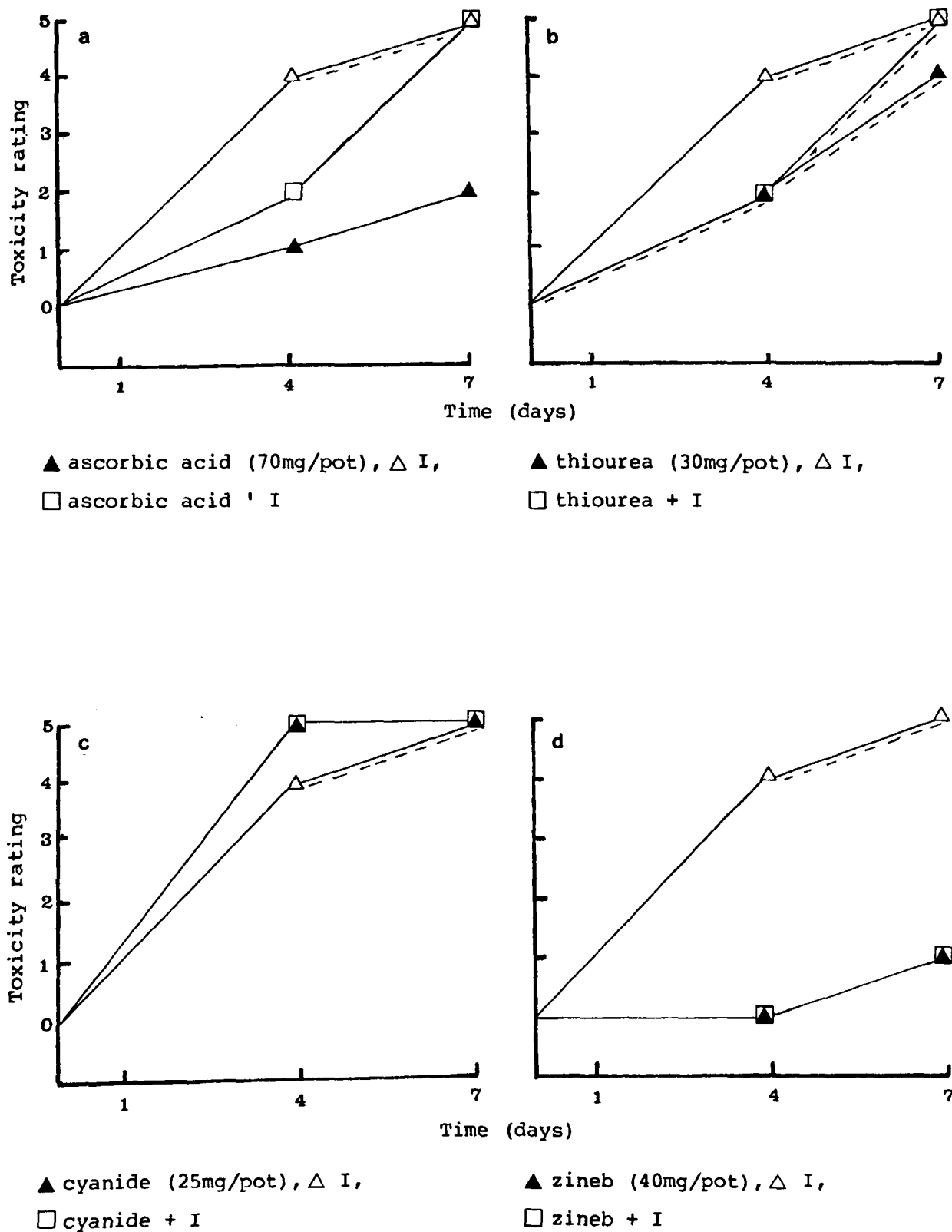
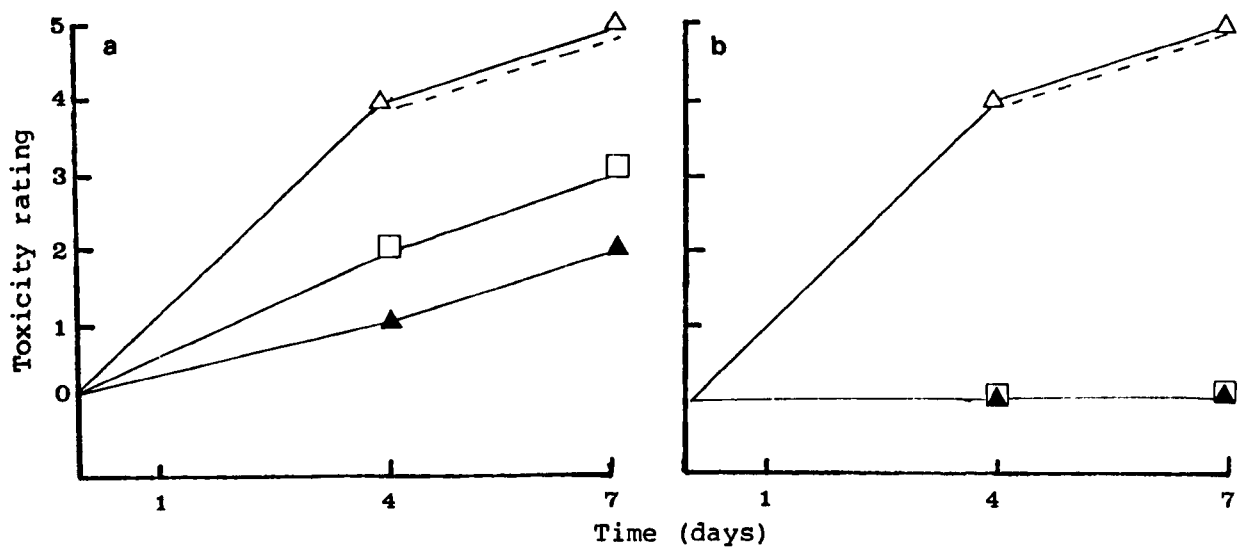


Figure 5.12 Influence of simultaneous addition of various compounds on the toxicity of I (2mg/pot) to dwarf French bean.

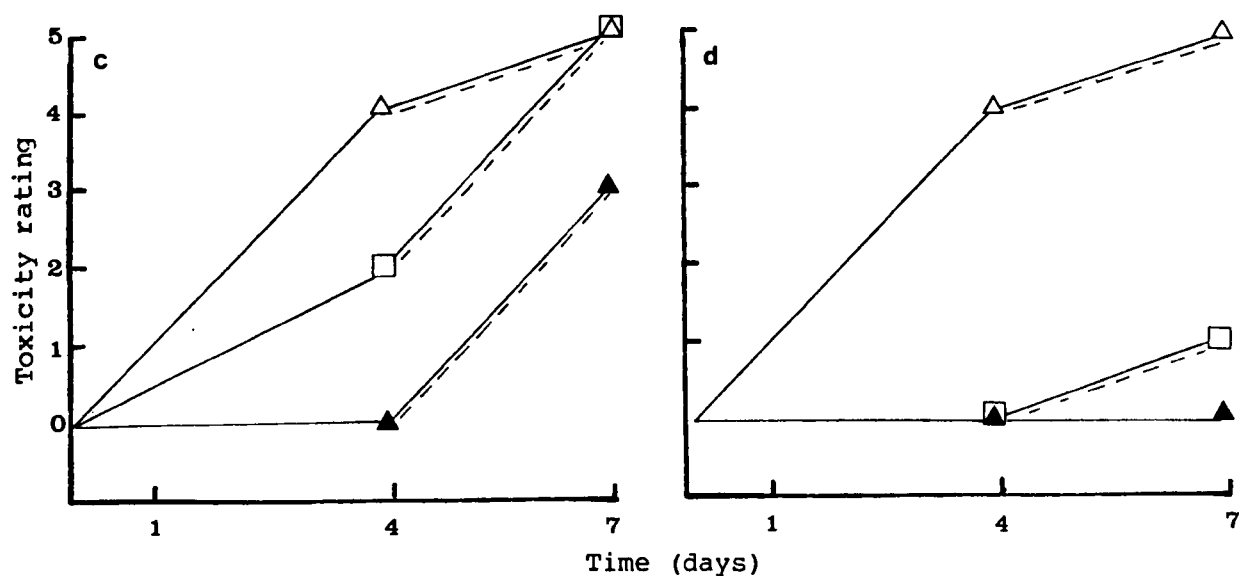


▲ ascorbic acid (100 mg/pot), △ I,

□ ascorbic acid + I

▲ zineb (20 mg/pot), △ I,

□ zineb + I



▲ thiourea (15 mg/pot), △ I,

□ thiourea + I

▲ thiourea (5 mg/pot), △ I,

□ thiourea + I

Figure 5.13 Influence of simultaneous addition of various compounds on the toxicity of I (2 mg/pot) to dwarf French bean.

possible protection against I toxicity. Some scorching of bracken leaflets by CN was observed earlier (see 4.3.1.2).

5.D.4 Discussion

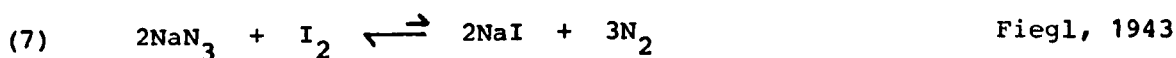
The pattern of inhibition of the I oxidation reaction by the additives employed in this study can be classified into five types:-

1. No effect on the reaction, e.g. allylthiocyanate, F, Cl and Br at 5 mM.
2. No reaction whatsoever, e.g. zineb, thiourea, CN, ascorbic acid and cysteine at 5 mM.
3. After an initial lag in the reaction, the rate and time span of reaction is the same as for I alone. The extent of I_3 absorption being lower, presumably due to the initial lag in reaction, e.g., zineb 0.25 mM, cysteine 1 mM.
4. After an initial lag in the reaction, the rate of reaction is slower, the time span longer and the I_3 level attained lower than for I alone, e.g., thiourea, CN and ascorbic acid at 0.25 mM.
5. No initial time lag, the rate of reaction slower, time span longer and I_3 level lower than for I alone, e.g., N_3 at 5 mM.

No additive produced the pattern of inhibition observed for SCN 0.4-10 mM (see figure 5.1), i.e., no initial time lag, rate slower, time span longer, I_3 level rising then falling away again.

The total inhibition observed (type 2) and the initial lag time with lower levels of additives (types 3,4) are almost certain to be due to the reduction by the additives of formed I_2 as previously suggested by

by Randall (1946) to be the reason for apparent peroxidase inhibition by thiols or sulphydryl groups. Ascorbic acid will also be oxidised by I_2 (Saunders et al., 1964) and CN will react rapidly with I_2 to form I and the cyanogen iodide ICN (Sneed et al., 1954). The instantaneous fall in I_3 level on addition of these chemicals helped to support this (figure 5.8). The slower decline with an addition of N_3 (figure 5.8) would correspond with no lag being produced on incubation with I (type 5). SCN, at an equivalent level, resulted in a more rapid decline than N_3 , although not nearly so rapid as the other additives, hence the observed reversal during the incubation reaction with I (figure 5.1). N_3 will react with I_2 according to the equation:



This reaction is very slow but is catalysed by traces of sulphide, thioketones and sulphydryl groups (Fiegl, 1943). It can only be assumed that with N_3 the rate constant for its reaction with I is lower than for the SCN reaction and this in turn is much lower than for the reaction of I with the other additives. No catalyst is required for the SCN/ I_2 reaction (equation 6 above) - Kozlovski, 1947; Lewis and Skoog, 1962.

Besides reaction with the formed I_2 , some true inhibition of the peroxidase enzyme by the additives can be expected, especially from those resulting in types 4 and 5. CN and N_3 are both well known peroxidase inhibitors by means of their strong reaction to the iron component of peroxidase (Keilan and Hartree, 1951). Ascorbic acid is oxidised by HRPO (Chance, 1949), thus competition for oxidising equivalents (compound 1) with I could be expected. Thiourea is not

oxidised by the HRPO system (Sorbo and Ljunggren, 1958) and there are conflicting reports on whether inhibition of peroxidase by thiourea does in fact take place (see Saunders *et al.*, 1964). Other than when iodometric methods have been used, cysteine has not been shown to exhibit any inhibitory properties on peroxidase (Saunders *et al.*, 1964) and neither is it a substrate for the enzyme (Ljunggren, 1957). No data as to the effect on peroxidase of zineb was found in the literature. Cysteine, ascorbic acid, CN and thiourea are all known to react with free radicals intermediates (Bielski and Gebicki, 1976; Pryor, 1976; Treinan and Hayon, 1976); these compounds may inhibit peroxidase action by such a mechanism.

Thiourea is known not to react with PABA-red, the product of the PABA oxidation by the HRPO/H₂O₂ system (Randall, 1946). As an aid to gauging the enzyme inhibitory effects of the additives, PABA oxidations were carried out (see 5.D.3.4). Here only ascorbic acid and cysteine showed type 2 reactions at 5 mM and type 3 at the lower levels tested; indicating reaction with the azo dye and little direct inhibition of the enzyme itself (figure 5.11). This is surprising for ascorbic acid, bearing in mind it is potentially co-oxidised (Chance, 1949). Perhaps the optimum conditions (e.g. pH, E₀) were not achieved here, or the ascorbate oxidation with PABA-red is very rapid. It does, however, tend to confirm that in the case of I oxidations cysteine and zineb (the latter exhibiting a type 1 reaction with PABA) are only effective on the I₂ formed, not the enzyme. CN and thiourea both showed type 5's for PABA in contrast to their type 2 for I. This confirms that thiourea and CN can exhibit true enzymic inhibition.

N_3 behaved as it did for I oxidations.

Allyl mustard oil has been reported as having an inhibitory effect on Japanese radish and sweet potato peroxidases (Kojima and Yazaki, 1956). The lack of inhibitory activity in the present study (type 1) may be a result of insolubility in the system. It would be expected, however, that reaction of allyl isothiocyanate with I_2 could take place and this may be the reason for these authors observed inhibitions. The failure of F to inhibit the I oxidation reaction is surprising in view of the relative similarity in inhibitory power to CN as reported by Keilan and Hartree (1951).

On the limited scale that the plant studies were carried out, it was found that by manipulating the levels of the additives employed (except CN) it was possible to reduce I toxicity, or in the case of zineb, remove it altogether.

Zineb is a contact fungicide active on leaf surfaces (Cremlyn, 1978). It is difficult to establish exactly how it functions since it is unstable and the chemical nature of the fungitoxic agent(s) is not known for certain (Corbett, 1974). On leaf surfaces, it is thought that it may hydrolyse in the presence of moisture to water soluble ethylenethiourea (Bontoyan and Looker, 1973) which affects mammalian thyroids (Graham et al., 1973). Further hydrolysis to ethyleneisothiocyanate has also been proposed (Cremlyn, 1978). These, and other possible breakdown products, are believed to be responsible for the fungicidal action of zineb by the chemical inactivation of important thiol containing systems in the fungal cell (Corbett, 1974). The present study involved root application and similar substances may form,

either in solution before uptake or once absorption has taken place. It is likely that these breakdown products, as well as zineb itself, will react with any I_2 encountered in the plant.

Ascorbic acid is a natural plant constituent. Conflicting growth regulatory properties have been reported for exogenously applied ascorbic acid (see Sebrell and Harris, 1967). Oxidation of ascorbic acid results in H_2O_2 formation and the toxic and sub-toxic effects on plants may be due to this (Åberg and Johansson, 1963). A reduction in endogenous ascorbate content in tomato plants has been observed on application of 4-100 ppm of KI (Hageman et al., 1942) presumably due to reaction with I_2 .

Thiourea has been linked with SCN in affecting various plant processes, e.g., on the breaking of dormancy (Mayer and Poljakoff-Mayber, 1963; Denny, 1928) and as a defoliant (Addicott and Lynch, 1957). Conversion to thiocyanic acid, which contains a sulphydryl grouping, has been stated as necessary for thiourea to exert its effect on dormancy (Bokarev and Satarova, 1957). The defoliant properties of SCN are discussed further in chapter 6.

The following can be drawn from the above series of experiments: SCN is capable of inhibiting the overall oxidation of I by peroxidase systems and thus affects the plants ability to form toxic I_2 . The levels of potential SCN found in resistant species are higher than in sensitive species, this could be contributing to the selectivity demonstrated by I in plants. The mechanism by which SCN acts may involve some true enzyme inhibition but the major influence is its

ability to reduce I_2 to I. Other inhibitory substances present in plants and some exogenous compounds may act in a similar manner. Simultaneous or pre-addition of SCN and other additives to I susceptible bean resulted in at least temporary resistance to I.

These points are discussed in more detail along with some broader implications in chapter 7.

CHAPTER 6

THE MODE OF ACTION OF IODIDE AND THIOCYANATE

6.1 INTRODUCTION

Observations in the previous two chapters led to an interest in the use and mode of action of SCN and I, and other related anions, when applied alone to plants. When used as additives in bracken bioassay and field studies, variation in toxicity within the halides and some pseudohalides were found. I and, to a lesser extent SCN, were found to often exhibit toxic symptoms on bracken foliage. Bromide and chloride showed no deleterious effects in bioassays (see chapter 4). Defoliation properties for I and SCN were also noted when applied at low levels to dwarf bean (see chapter 5).

The mode of action of SCN as a herbicide in its own right has received very little attention although it has long been recognised as possessing contact herbicidal properties (Robbins et al., 1942; Brian, 1976). Several workers have reported hormonal effects which they have attributed to SCN. Swets and Addicott (1955) showed that NH_4SCN caused a rapid decrease in IAA levels in the leaves of Phaseolus vulgaris L. and induced leaf abscission. It has been claimed in parallel with ethylene to induce sprouting of dormant potato tubers (Denny, 1928 ; Ranjan and Kaur, 1954) and in fruit ripening (Hartman, 1959). Ethylene production by cotton leaves following application of NH_4SCN has been demonstrated (Jackson, 1952). Under normal growing temperatures Novikov and Barannikova (1954) found that $10^{-4} \text{ M } \text{NH}_4\text{SCN}$ stimulated root formation on

the stems of bean and geranium by 500%, whereas, at lower temperatures this level killed the plants.

The herbicidal properties of I have been studied in more detail (Mynett and Wain, 1971, 1973). These authors demonstrated that I toxicity depends on its oxidation within the plant to I_2 which is the more toxic agent, causing inhibition of the Hill reaction of photosynthesis and chlorophyll loss (see also chapter 5). Herrett et al. (1962) showed that low doses of KI induced leaf abscission in P. vulgaris while higher doses caused rapid dessication of the leaves which became "frozen" to the stem. They suggest an intimate relationship exists between I induced abscission and IAA levels within the affected leaves. Umaly and Poel (1970) demonstrated increased growth rates using low levels of KI and decreased growth with higher levels, again indicating a possible auxin involvement. Inhibition of the phototropic response of maize seedling coleoptiles by KI has been observed, although this has been reported to be through the reaction of I with the flavin photoreceptor (Schmidt et al., 1977). At all levels employed by Herrett et al. (1962), no effect was observed for Cl, Br or F on P. vulgaris.

The object of this study was hence two-fold:

- (1) To reaffirm the relative toxicities of the halides to dwarf bean as found by Herrett et al. (1962) and to compare them directly with the effects found by Swets and Addicott (1955) for the pseudohalide SCN.
- (2) To carry out specific test tube exercises on the effect of halides, SCN and some of their oxidation products in plants on various systems containing IAA in an attempt to account in chemical terms for the behaviour of these anions when applied to plants.

6.2 EXPERIMENTAL

6.2.1 Materials

Horseradish peroxidase (HRPO) - see also 2A - and riboflavin were purchased from the Sigma Chemical Company, London. IAA was purchased from the Aldrich Chemical Company Ltd. All other reagents were purchased from either British Drug Houses Ltd. or Hopkin and Williams Ltd. and were of the purest grades available. Reacti-vials (5ml) were purchased from the Pierce Chemical Company.

6.2.2 Methods

6.2.2.1 Plant toxicity studies

Dwarf French beans (Phaseolus vulgaris L. c.v. Canadian Wonder) were germinated in trays of vermiculite maintained in a growth room at $30 \pm 3^\circ\text{C}$ with a 16h day length. Seedlings were selected on the basis of uniformity of size and lack of damage. These were transplanted into 10 cm pots for a foliar application study and small pots without drainage holes containing 13g of vermiculite for a root application study.

The influence of foliar applications of the sodium salts of the halides - I, Br, Cl and F and the pseudohalide SCN were assessed as follows. The plants were sprayed to run off with 10^{-3} , 2×10^{-2} and 10^{-1} M solutions of the various salts under test. After much preliminary work these concentrations were selected in order to clearly demonstrate the effects of the ions under test and to aid in presentation. The vermiculite was covered in cotton wool to prevent contamination of the vermiculite and the possibility of root uptake. Three plants per treatment level were employed. After spraying they were transferred to

a Fisons growth cabinet (see 2.2.2.1) at $25 \pm 0.75^\circ\text{C}$ and approximately 100% r.h. for 24h to aid uptake. Thereafter, humidity was reduced to $70 \pm 5\%$ r.h. The results were visually assessed over an 8 day period on an arbitrary 0 to 5 scale as before (see 5.A.2.5) depending on the degree of wilting or dessication (0 = no effect, 5 = severe wilting or dessication).

For the root application study NH_4SCN and NaI were applied to the vermiculate at rates of between 0-30 mg/pot and 0-6 mg/pot, respectively in a total volume of 100 ml and the plants watered as necessary thereafter. These levels were chosen in accordance with the observations of 5.B.1. These plants were also maintained in a growth cabinet at $25 \pm 0.75^\circ\text{C}$ and $70 \pm 5\%$ r.h. with a 16h day length for the duration of the experiment and assessed as previously described.

In both studies the plants were treated at the stage when the primary leaves were fully expanded and the first trifoliate leaves were beginning to develop.

6.2.2.2 Enzymic IAA oxidation study

IAA was reacted at 37°C in the presence of (I) NaI alone, (II) H_2O_2 alone, (III) NaI and H_2O_2 , (IV) HRPO , (V) HRPO and NaI , (VI) HRPO and H_2O_2 , (VII) HRPO , NaI and H_2O_2 . IAA was also reacted in a similar manner with NaCl , NaBr , NaF or NaSCN . The reaction was terminated after 1h by taking a 2 ml aliquot and adding this to 2 ml of 0.05M NaAsO_2 . The remaining IAA was determined by the method of Tang and Bonner (1947) after acidifying, extracting into ether and re-extracting into 0.1M NaHCO_3 to remove the halide or SCN . This extraction

procedure was found necessary due to the interference in colour formation from NaI and NaSCN and so for uniformity of procedure was carried out in all cases. The volumes and concentrations of reagents were as follows: HRPO-0.5 ml of 0.5 mg/ml, H_2O_2 -0.1 ml of 44 mM, halide or pseudohalide-2.5 ml of 8 mM, IAA-6 ml of 0.6 mM. Both the HRPO and halide or SCN were made up in phosphate buffer, pH 6.0, 0.04M. If either of these reactants was not in the reaction mixture it was replaced by the equivalent amount of buffer.

6.2.2.3 Riboflavin photosensitized IAA oxidation

The reaction mixtures were constantly shaken in 50 ml volumetric flasks under fluorescent lighting for the duration of the experiment (1.5h). These reaction mixtures consisted of IAA-3.6 ml of 2 mM in phosphate buffer (0.1M, pH 7.2), riboflavin solution-2 ml of 0.1mg/ml and halide or SCN- 3.6×10^{-5} moles (5:1 M/M halide or pseudohalide to IAA). All reactions were carried out in duplicate. On completion of the experiment, the reaction mixtures were made to 50 ml and 3 ml aliquots withdrawn. Extraction and IAA determination were carried out as previously described (6.2.2.2).

6.2.2.4 Chemical oxidation of IAA by thiocyanogen and iodine

The IAA was dissolved in 10% v/v methanol in carbon tetrachloride (10mg/100ml). I_2 was dissolved in carbon tetrachloride (20mg/100ml). Thiocyanogen- $(\text{SCN})_2$ -was prepared by the method of Gardner and Weinberger (1939) and diluted with carbon tetrachloride to give a $(\text{SCN})_2$ concentration of 29mg/100ml, assuming a quantitative conversion from the SCN anion.

The reactions were carried out under fluorescent lighting and in darkness in 5 ml reacti-vials with constant stirring for the duration of the experiment (4h). The reaction mixtures consisted on 2 ml of I_2 or $(SCN)_2$ solution and 2 ml of IAA solution. On completion of the experiment 2 ml aliquots were withdrawn and the residual IAA immediately extracted into 4 ml of 0.1M $NaHCO_3$ and determined as described in 6.2.2.2. Appropriate standards and blanks were also incorporated.

In addition, similar concentrations of IAA in aqueous solution were reacted with NaI, NaCl, NaBr, NaF and NaSCN (at equimolar concentration to the I_2) in a total volume of 4 ml for 4h in reacti-vials under fluorescent lighting. On completion, 2 ml aliquots were acidified, the IAA extracted into ether, re-extracted into 0.1M $NaHCO_3$ and determined as previously described.

In all cases the reactions were carried out in duplicate.

6.3 RESULTS

6.3.1 Plant toxicity studies

The results of the foliar application study were generally in agreement with those of Herrett et al. (1962) for the halides and those of Swets and Addicott (1955) for NaSCN. NaCl and NaBr had no influence on growth at any of the concentrations tested while NaF brought about slight scorching of the primary leaf margins at the two highest concentrations. NaI brought about wilting and defoliation of the primary leaves at the two lowest levels while at the highest level, rapid wilting and dessication was brought about. However, no defoliation was observed.

Herrett et al. (1962) referred to this as the leaves being "frozen" onto the stem. At the lowest NaSCN concentration tested, no effect was observed, at the intermediate level slight wilting was observed, while at the highest level, the leaves were again "frozen" onto the stem. This would seem to suggest that levels which would bring about defoliation might be found between these latter two (Figure 6.1).

Because of difficulties in obtaining uniform effects with reasonable trends over a range of SCN and I concentrations, probably because of uptake problems, the application method was changed from foliar to root. The results of this study revealed that at low concentrations, 3 and 30 μg per pot, NH_4SCN had no effect on growth while at 300 μg and 3 mg per pot, wilting followed by defoliation was observed. At 30 mg per pot "freezing" occurred. With NaI, slight wilting was observed at 6 μg per pot while at 60 and 600 mg per pot, wilting and defoliation were observed. At 6 mg per pot "freezing" occurred (Figure 6.2).

6.3.2 Enzymic IAA oxidation study

The results of this study indicated that in the absence of NaI, 69.1% of the IAA was destroyed by HRPO. In its presence, this was reduced to 6.4%. However, in the presence of NaI and H_2O_2 , destruction was increased to 90.9%. In the absence of HRPO, NaI alone and H_2O_2 alone had no effect (0.0% IAA destruction) while in combination, 13.2% destruction was brought about.

For the NaSCN experiment, IAA destruction in the presence of HRPO was 74.4%. In the presence of NaSCN and HRPO, this was reduced to

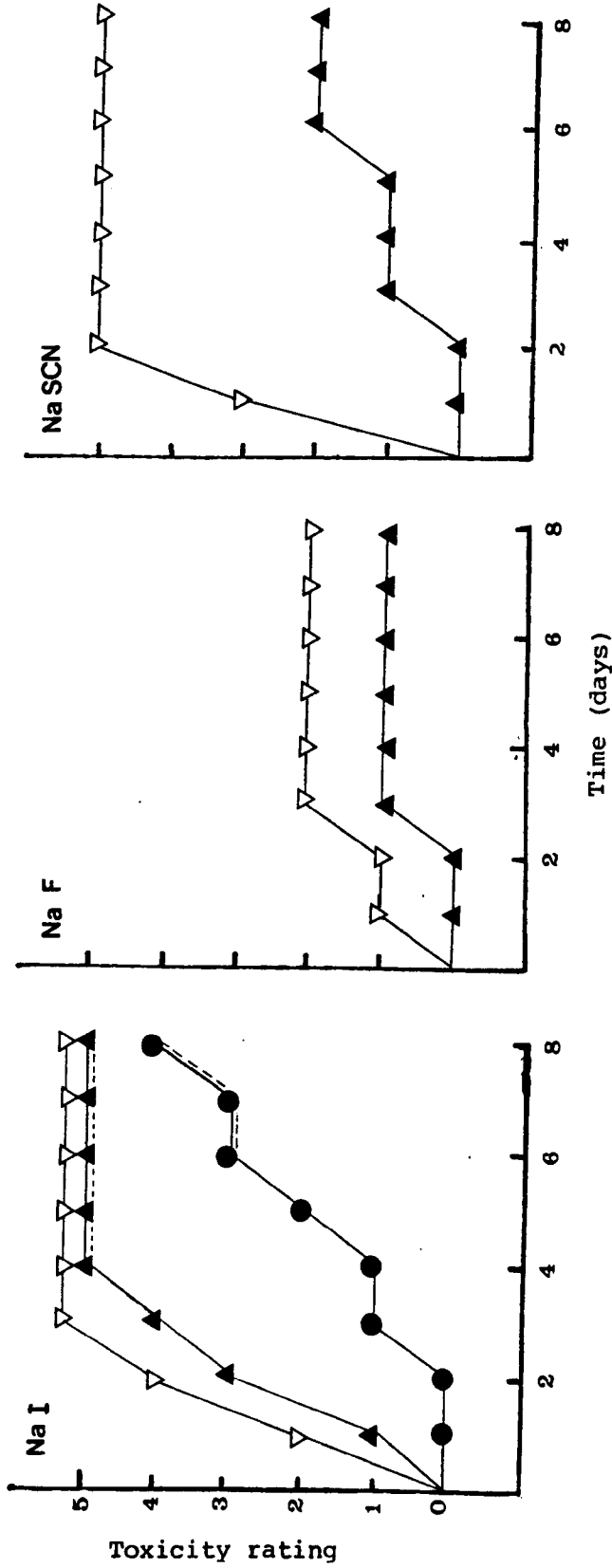


Figure 6.1 Comparison of the toxic effects of foliar applications of NaI, NaF and NaSCN on dwarf French bean. Concentration of spray solutions: $10^{-3}M$ (●), $2 \times 10^{-2}M$ (▲), $10^{-1}M$ (▽). ----- indicates some defoliation occurring.

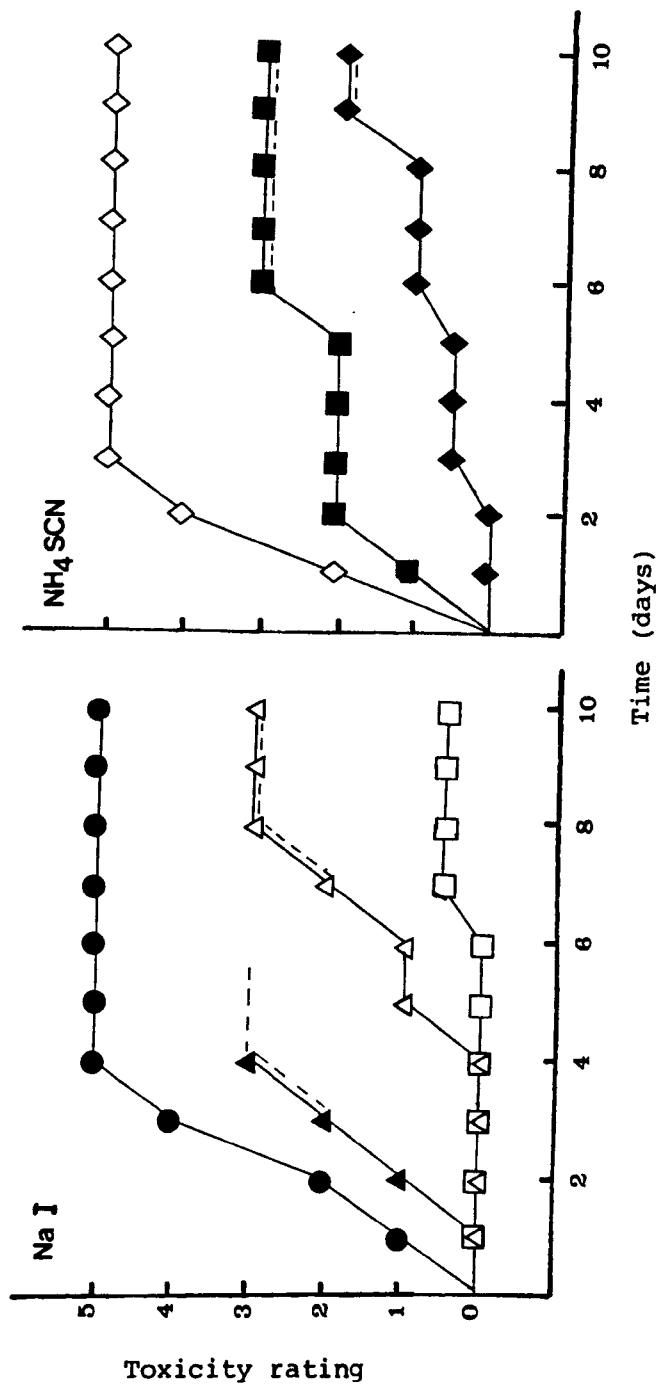


Figure 6.2 Influence of root application of a range of NaI concentrations (0-6 mg per pot) and NH₄SCN (0-30 mg per pot) on wilting and defoliation of dwarf French bean. NaI: 6µg per pot (◻), 60µg per pot (△), 600µg per pot (▲), 6 mg per pot (●). NH₄SCN: 300µg per pot (◊), 3 mg/pot (◼), 30 mg/pot (◊). ----- indicates some defoliation occurring, ----- indicates 100% defoliation.

53.5% and further reduced to 45.6% by NaSCN, H_2O_2 and HRPO. In the absence of HRPO, NaSCN and NaSCN plus H_2O_2 had no effect on IAA. Similar patterns of response were observed for NaBr, NaCl and NaF although the tendency for the halide to inhibit IAA destruction progressively decreased in that order (Figure 6.3).

6.3.3 Riboflavin photosensitised IAA oxidation

NaI and NaSCN reduced IAA destruction from 95.7% to 25.5 and 24.6% respectively. None of the other halides had any effect on oxidation (Table 6.1).

Table 6.1 Influence of sodium halides and sodium thiocyanate on the riboflavin photosensitised oxidation of IAA.

Reaction mix	% IAA oxidation
IAA	0.0
IAA + riboflavin	95.7 (6.1)
IAA + riboflavin + NaI	25.4 (3.0)
IAA + riboflavin + NaSCN	24.6 (4.2)
IAA	0.0
IAA + riboflavin	92.5 (1.1)
IAA + riboflavin + NaBr	88.4 (2.3)
IAA + riboflavin + NaCl	94.2 (3.5)
IAA + riboflavin + NaF	94.2 (3.5)

Values are means of two replicates. Figures in parentheses are standard deviations (n-1 D.F.)

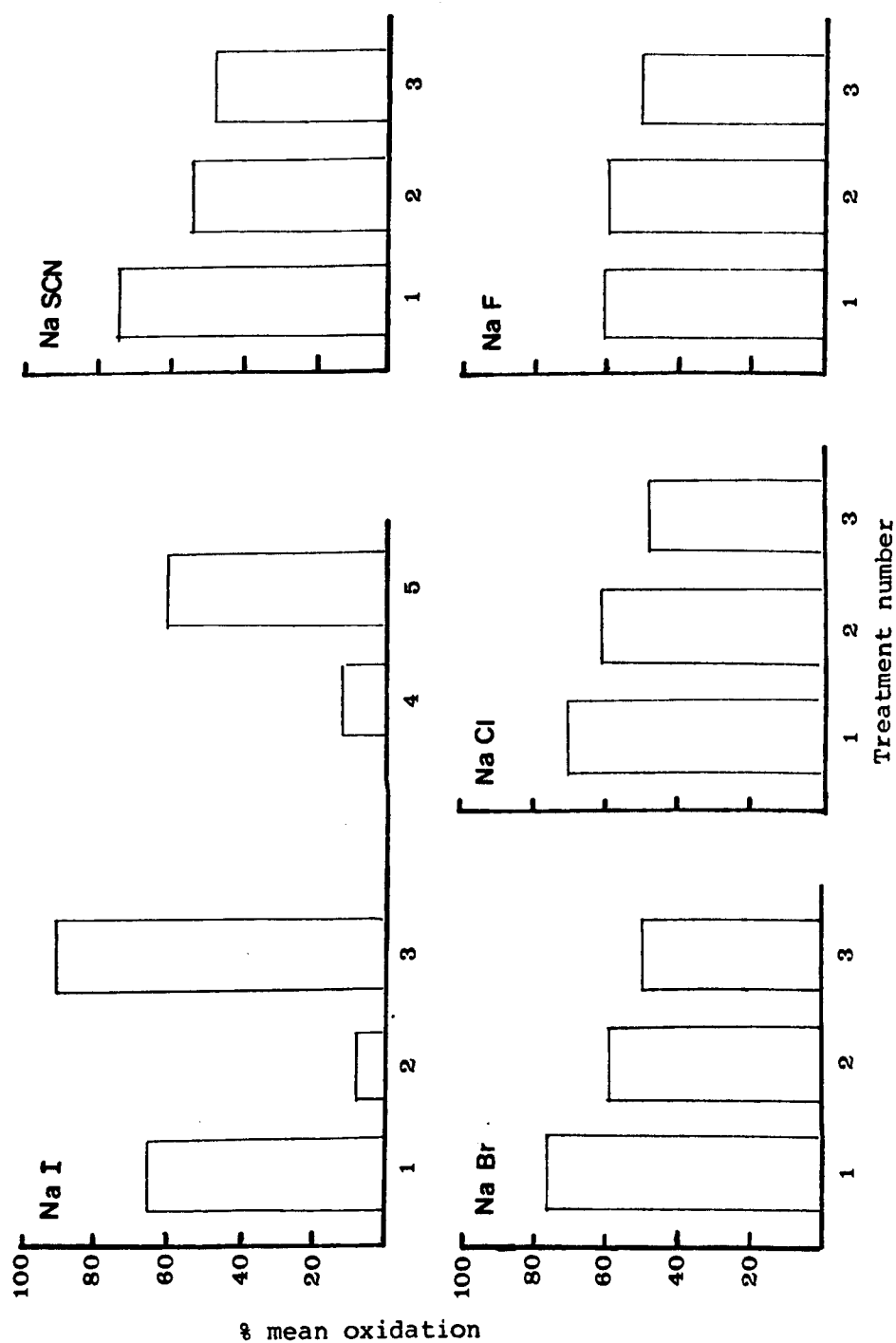


Figure 6.3 Influence of NaI, NaSCN, NaBr, NaCl and NaF on the oxidation of IAA by HRPO. Treatment numbers: (1) IAA + HRPO, (2) IAA + HRPO + salt, (3) IAA + HRPO + salt + H_2O_2 , (4) IAA + NaI + H_2O_2 and (5) IAA + HRPO + H_2O_2 .

6.3.4 Chemical oxidation of IAA by thiocyanogen and iodine

IAA alone was not influenced by the reaction conditions over the timescale of the experiment. However, in the presence of I_2 in the light, 51.8% was destroyed, while in the dark, this was reduced to 21.4%. In the presence of $(SCN)_2$, 85.0 and 92.9% destruction was brought about in the light and dark respectively (table 6.2). IAA under fluorescent lighting, in the presence or absence of the halide or pseudohalide, was completely unaffected.

Table 6.2 Influence of iodine and thiocyanogen on the destruction of IAA.

Treatment	% oxidation
IAA (light)	0.0
IAA (dark)	0.0
IAA + I_2 (light)	51.8 (2.5)
IAA + I_2 (dark)	21.4 (2.5)
IAA (light)	0.0
IAA (dark)	0.0
IAA + $(SCN)_2$ (light)	85.0 (0.7)
IAA + $(SCN)_2$ (dark)	92.9 (0.0)

The level of $(SCN)_2$ employed was x3.2, in molar terms, than that of I_2 , assuming quantitative synthesis of the pseudohalogen. Values are means of two replicates. Figures in parenthesis are standard deviations (n-1 D.F.).

6.4 DISCUSSION

The results of this investigation have reaffirmed that of the halides and pseudohalide SCN, only I and SCN have defoliant properties while direct comparison of these two indicated very similar toxic symptoms. Both caused chlorosis and defoliation at relatively low levels and rapid dessication and freezing at higher levels. I was however more toxic. Additionally, the mode of action of both has been shown to be closely linked to IAA metabolism (Swets and Addicott, 1955; Herrett et al., 1962).

Foliar applied SCN is reported to be translocated from primary leaves to the trifoliates of dwarf bean, where it also exhibits its toxic properties (Swets and Addicott, 1955). I damage is largely confined to leaf to which it applied (Herrett et al., 1962; Mynett and Wain 1971). These trends were followed in the present study. I is said not to move because of a lack of phloem movement or as a consequence of biochemical retention within the treated area and a rapid toxic effect (Herrett et al., 1962). Root applied I translocates throughout the growing plant (Herrett et al., 1962).

Toxic properties similar to those found by Herrett et al. (1962) were observed for F. No oxidation of F by peroxidase has been reported and chemical oxidation within the plant is unlikely ($E_o + 2.65v$) (Latimer, 1952). It seems probable that the observed ion effects are due to the halide anion. It has been reported that respiratory enzymes, in particular enolase (EC 4.2.1.11), are sensitive to F (Miller and Miller, 1974). F has also been reported to inhibit inorganic

pyrophosphatase (EC 3.6.1.1) and hence the oxidation of free fatty acids (Lehninger, 1975). Hara et al. (1977), studying the long term effects of halide application to cabbage, also found F toxic. They found the halides to be toxic in the same order as the present study, $I > F > Br > Cl$. The accumulation was greater for Br and Cl than for I and F. Yuita et al. (1975) stated that their absorption and translocation in rice was also in this order. This further supports the notion that although mobility and accumulation are important, the ability to be oxidised to toxic halogen will be more vital, F being somewhat an anomaly - not being oxidised.

When HRPO was used both as an oxidase and a peroxidase, the particular functions of I were readily noted:

- 1) In the absence of H_2O_2 , IAA destruction was prevented, possibly due to the free radical scavenging properties of I (Bellus, 1978) or to a more general inhibition of peroxidase action as discussed for SCN previously (chapter 5).
- 2) In the presence of H_2O_2 enhanced destruction of IAA was noted compared with control samples. This can be explained in terms of the production of I_2 from I, a recognised metabolic pathway (Mynett and Wain, 1971, 1973) - see also chapter 5. In turn, Morrison and Bayse (1973) have shown that I_2 will oxidise tryptophan yielding an oxindole derivative. Similarly, the oxidation of IAA by Br_2 has also been demonstrated by Hinman and Bauman (1964). This reaction with indoles can also be accomplished with other halogens including F_2 and Cl_2 (Powers, 1972) and has been demonstrated in the present study with the pseudohalogen $(SCN)_2$ and IAA.

When SCN was submitted to the above sequence of events only the scavenging or peroxidase inhibitory role was demonstrated in that IAA was protected to some degree in both the presence and absence of peroxide. This was in line with the previous findings (chapter 5), and those of Sorbo and Ljunggren (1958) and Chung and Wood (1970), that SCN is not oxidised by HRPO. Therefore, although the peroxidase system could account for the behaviour of I in destroying IAA in plants it is inadequate as an explanation for SCN, placing the latter more in line with Br and Cl which does not meet the facts. Attempts to influence IAA destruction in the riboflavin photosensitised system again illustrated the ability of I and SCN to scavenge free radicals and protect IAA in a non-enzymic situation.

Faced with this situation, attention was turned to a consideration of the role of halogens in plants. Cl has been shown to be essential in that it acts as an electron acceptor in the splitting of water by photosystem II, thereby releasing oxygen (Bové et al., 1963; Heath and Hind, 1969). In the absence of Cl irreparable damage occurs to the photosystem, the inclusion of Br will partly prevent this (Hind et al., 1969). The inefficiency of the latter is partly based on size, but more so on its instability in that it is slowly oxidised to Br₂ which is toxic. I is totally unacceptable due to its rapid oxidation to I₂. The behaviour of these three halides can be explained in terms of their oxidation potentials (E₀) (Hewitt, 1979).

Cl (E₀ + 1.3v) is non toxic and mobile, Br (E₀ + 1.08v) can be slowly oxidised to bromine and I⁺ (E₀ + 0.60v) is rapidly oxidised to I₂. The oxidation products once formed can, amongst other things, bring about

the rapid destruction of IAA (see above). On this basis it can be predicted that SCN will be oxidised to $(\text{SCN})_2$, as its oxidation potential ($-E_0 + 0.77\text{v}$ - Latimer, 1952) is intermediate between I and Br, leading to destruction of IAA in a similar manner to the other halogens.

In chemical terms, $(\text{SCN})_2$ will behave essentially in the same way as the halogens, although some problems can arise due to the relative instability of the reagent under physiologically acceptable conditions (Guy, 1977). From studies carried out with proteins (Adams et al., 1972), $(\text{SCN})_2$ at low concentrations was shown to exhibit a high degree of selectivity for the indole nucleus (as tryptophan). This marked selectivity was not shown by the halogens.

Without denigrating the enzymic approach as a mechanism for accounting for I - IAA interactions in plants, it would appear that photochemical oxidation via the photosynthetic apparatus could possibly suffice in accounting for the phytotoxic properties of I and SCN. The fact that SCN is required in much higher concentrations (X5-X10) than I before herbicidal effects are recognised (figure 6.2) is in line with their ease of oxidation, chemical and enzymic. Accumulation at the active sites needs also to be considered here and the fact that SCN may be isomerised to isothiocyanates or bound up in glucosinolates. The need for higher concentrations and the marked selectivity of the SCN - IAA interaction in contrast to I could make the use of the former as a mechanism for controlling IAA levels in plants particularly attractive. Of course, at higher concentrations of SCN and I other non-specific interactions (see below) will undoubtedly occur which may account for the fact that at low levels hormonal effects due to the influence on

IAA are easily recognisable, whilst at higher concentrations of chemical more general "freezing" effects occur.

Other types of SCN or I - IAA interactions are possible. The exact mechanism of how IAA controls plant growth is a subject of great debate and is considered beyond the scope of this study. However, it would seem reasonable to assume that factors affecting IAA levels will upset its normal functional role in plants. The present study has shown that the effect of in-vivo produced (SCN)₂ or I₂ on IAA could be responsible for their observed growth regulatory effects. One theory of IAA mechanism of action is that the active agent is its IAA-oxidase reaction product 3-methyleneoxindole (Still et al., 1965; Moyed and Tuli, 1968). The biological activity of this compound in higher plants is attributed to its ability to react rapidly with sulphhydryl groups, including sulphhydryl enzymes, to form thiol esters (Still et al., 1965; Moyed and Tuli, 1968). 3-Methyleneoxindole, like other sulphhydryl reagents, is thus capable of releasing regulatory enzymes from sensitivity to feedback control (Tuli and Moyed, 1966; Moyed and Tuli, 1968), thus having the potential for accelerating metabolism at relatively low concentrations and inhibiting at high concentrations due to more general effects on metabolism (Moyed and Tuli, 1968). This could result in an auxin type growth pattern, i.e. stimulation at low levels and inhibition at high levels. The oxidation of thiol groups by I₂ is well known (Smythe, 1909; Jocelyn, 1972). The pseudohalogen (SCN)₂ is also a sulphhydryl reagent (Hughes, 1975; Aune et al., 1977). The possibility thus exists that the in-vivo auxin-type activity of I and SCN may result from activity similar to that postulated for 3-methyleneoxindole on SH

groups. Again prior oxidation to the halogen or pseudohalogen would be necessary.

The levels of endogenous thiol containing substances have been found to be important around the time of the breaking of dormancy (Guthrie, 1937; 1937a), early growth (Hopkins and Morgan, 1943; Jocelyn, 1972) and in times of plant stress (Fedtke, 1981). Exogenous applied ethylene chlorohydrin (an ethylene generator), NH_4SCN (Denny, 1928; Ranjan and Kjaer, 1954) and thiourea (Denny, 1928) have been found to result in a loss of dormancy in potatoes. An increase in the levels of SH containing substances, especially glutathione, and ascorbic acid was found for ethylene chlorohydrin (Guthrie, 1937; 1937a). The activity of NH_4SCN here has been attributed to its conversion to thiocyanic acid which itself possesses a SH group (Bokarev and Satarova, 1957). The addition of neutralised IAA inhibited the breaking of dormancy, the sprout growth and increase in SH content in potatoes treated with ethylene chlorohydrin (Guthrie, 1937b). Thus, although Guthrie's IAA was probably acting against the effect of ethylene, the in-vivo effect of SCN in the present study may be a result of its conversion to thiocyanic acid and a metabolic balance between sulphydryl content and IAA. It should be remembered that an actual drop in IAA levels was measured by Swets and Addicott (1955) in defoliating bean leaves which would be more likely to result from a $(\text{SCN})_2$ -type reaction. It should also be remembered, of course, that the control of abscission is a complex function of plant hormones (including IAA and ethylene) and nutrients acting near or distant from the abscission zone. The influence of a regulating chemical to modify one or more of these actions

or interactions is necessary for defoliant properties to be manifested (Addicott, 1976). The lowering of IAA is only one of the factors involved although it is considered to be of major importance (Carns, 1966; Addicott, 1976). The activity of thiourea on potato dormancy and its activity as a defoliant (see 5.B.3) may be due to its isomerisation to NH_4SCN (Bokarev and Satarova, 1957) .

On considering the mechanism of I_2 toxicity at high levels of toxicity, iodination of plant components following treatment can be expected. The integrity of a great many SH compounds is necessary for biochemical processes to function normally in plants (Jocelyn, 1972). Their oxidation by I_2 (see above) could be important. Reaction of I_2 with unsaturated compounds present within leaves (Mynett and Wain, 1971), in particular proteins (Ramachandran, 1956) is probable. I_2 forms a 1:1 charge transfer complex with chlorophyll a in-vitro (Livingston and Conrad, 1969). This combination may affect the electronic configuration of chlorophyll in the light reactions, resulting in the observed inhibition of the Hill reaction (Mynett and Wain, 1973). Chlorophyll a destruction in-vivo has also been demonstrated (Mynett and Wain, 1973). Reactions with chloroplasts through protein complexing and iodination, resulting in inhibition of photosynthesis, have been reported (Canellakis and Akoyunoglova, 1976). These authors showed that inhibition of photosystem II of photosynthesis predominates and that the iodinating species is perhaps not an enzyme/I complex, but some form of free-radical I. It has already been discussed that I will be oxidised by this photosystem in-vivo; free radical intermediates are possible here. This again indicates the importance of the chemical oxidation of I, in

addition to its peroxidase catalysed reaction, in I mode of action.

Bearing in mind the relative instability of $(\text{SCN})_2$ (Guy, 1977), it could be expected that, if SCN is applied at a level high enough and sufficient $(\text{SCN})_2$ produced, this pseudohalogen would behave similarly to iodine against plant components. In-vitro oxidation of chlorophyll a by $(\text{SCN})_2^-$ radicals and Br_2^- radicals has been reported (Chauvet et al., 1979). Wu and Basler (1969), using chloroplasts isolated from cotton cotyledons, demonstrated rapid inactivation of these plastids by NH_4SCN which could be indicative of $(\text{SCN})_2$ toxicity.

The effects of the ions themselves cannot wholly be ruled out. Mynett and Wain (1973) found I ion itself inhibited the Hill reaction in isolated P. vulgaris chloroplasts, albeit by the order of $\times 10^4$ less toxic than I_2 . This was considered to be either a true effect of the ion or again may follow the photo-oxidation in-vivo of a small quantity to I_2 via the photosynthetic apparatus. SCN and I ions are examples of chaotropic agents which are said to interrupt the hydrogen bonding of water, thereby making hydrophobic regions of membranes more accessible (Hatefi and Hanstein, 1969). Yao et al. (1972) found structural alterations, mainly swelling and membrane separation, of the isolated chloroplasts of spinach leaves, in the presence of I and SCN. This was considered to be due to their properties as chaotropic agents. Lozier et al. (1971), again with isolated spinach chloroplasts, demonstrated inhibition of photosynthetic electron transport between water and photosystem II by chaotropic agents, including NaSCN. These inhibitory treatments resulted in manganese release from the chloroplast. The effects on photosynthesis noted by Wu and Basler (1969) may thus have been due to these chaotropic effects or from $(\text{SCN})_2$ toxicity.

CHAPTER 7

CONCLUSIONS

The role of this chapter was to draw some conclusions as to the outcome of the studies. Since time did not permit the continued study of many of the aspects covered, some suggestions for further investigations are made.

The overall aim of this thesis was the improvement of effectiveness of herbicides for bracken control. If a strictly practical point of view was adopted then this aim has not been met in that none of the approaches adopted have resulted in significantly improved bracken control. However, from a more scientific viewpoint it is felt that most of the individual aims have been, at least, partially, met. A contribution to the bank of knowledge on the physical, chemical and biochemical behaviour of the herbicides in some plant and soil systems has been made and some success has been achieved in modifying their behaviour, under certain circumstances, by the use of additives. It is believed that, with some further work, several of the avenues followed will eventually lead to improved field performances, not only in the use of the chemicals on bracken but also in general weed control situations.

The aim of chapter 2 was to look at the mechanism of action of asulam in plants to gain information as to the reasons for its success as a herbicide and its observed selective action as such. As in the text, initial in-vitro enzyme assays and plant growth studies concerning auxin-type behaviour of asulam led into interesting aspects involving

sulphonamide behaviour for asulam on which the study then concentrated. With a series of plant growth studies, a rationale for the mechanism of action of asulam in plants was then built up.

The studies showed that the biochemical mechanism of action of asulam almost certainly involves its activity as a sulphonamide compound, inhibiting the incorporation of PABA into folic acid by 7,8 dihydrofolate synthetase, leading to impairment of the synthesis of purine bases and resulting in reduced protein and nucleic acid synthesis. Since the completion and publication of the main part of this work (Stephen et al., 1980), several independent papers have been published which substantiate these findings (Killmer et al., 1980; Veerasekaran et al., 1981; 1981a). Biochemical aspects of the work have been carried on in this laboratory by Mrs. B.R. Kidd, conclusive effects on in-vivo purine nucleotide biosynthesis being found (see Kidd et al., 1982).

Particular outstanding questions remaining to be answered concern the role of PABA in plants and the possibility of auxin involvement in asulam's mechanism of action. The notion stated herein that plants with a higher content of folates may be less susceptible to asulam has been confirmed by Veerasekaran et al. (1981a). The partial selectivity of asulam in bracken control (see 1.4) may be a result of differential folate levels in bracken and the various grass species concerned. As was mentioned earlier, the findings of the present study on the application of PABA/asulam combinations may provide potential for broadening the selectivity of asulam in weed control in general. This may also be appropriate for decreasing sward damage by asulam in bracken control. A good deal of further work would be

necessary to assess the value of such combinations.

Although with an aim to investigate the possible pre-emergence activity of asulam for bracken control, the work of chapter 3 developed more into a study on the use of surfactants and other additives to regulate the behaviour of asulam in soil. The idea was to improve the chances of control between applied asulam and the rhizome buds in view of the circumstances resulting from the soil properties of asulam and the nature of the soils often found under bracken. In the event, pre-emergence asulam did not have any effect on bracken control at the rate applied and on the particular site used. Suggestions were made for a wider study using a range of concentrations and on different, possibly less difficult, sites.

The use of 1% cetab or 1% SDBS in the spray formulations in the field had no beneficial effect on control, although in laboratory studies the surfactants employed had achieved significant effects on asulam adsorption and leaching. Despite the interesting laboratory results, it was concluded that the use of surfactants to achieve flexible control over the movement of asulam and other pesticides in field would be of limited value. The reasons for this are explained in 3.3.3. Any future work here would probably involve low organic matter soils and situations where fairly specific surfactant - or additive - pesticide combinations were taking place. No additive treatment (surfactant or carbamate-type compound) which would lengthen the degree of persistence of asulam was found.

The work of chapter 4 illustrated in field and laboratory bioassays the importance of the concentration of the scavenger anion SCN in reducing the degree of detoxification of aminotriazole by a free radical mechanism. Field results, using a range of NH_4SCN to aminotriazole concentrations, revealed that the 1:1 molar ratio used commercially on bracken may not be optimum for all conditions; reduced aminotriazole and SCN scorching of the fronds and reduced grass damage due to the additive could be achieved by manipulating the SCN concentration. Similar conclusions were drawn from the bracken pinnule assessment method developed to rapidly assess the effect of SCN and other additives on aminotriazole scorching and additive phytotoxicity. The degree of aminotriazole scorching is dependent on the free radical generating capacity within the plant and this determines the optimum amount of SCN necessary for aminotriazole protection.

Aminotriazole formulations may also be tailored for uses other than bracken, as shown by preliminary investigations using dock and potato leaves. The degree to which a free radical reaction mechanism may contribute to the detoxification of other herbicides in bracken and other plants is also worthy of further investigation. The possibility of asulam breakdown by such a mechanism and the use of SCN in asulam formulations was discussed. Studies carried out in this laboratory (Al-Jaff, 1982) have shown that 2,4-D will undergo oxidation by in-vitro free radical generating systems and that these reactions are inhibited by SCN and other free radical scavengers. The phenoxyacetic acid herbicides (e.g. 2,4-D, MCPA, 4-CPA etc.) have been used as systemic herbicides for bracken control with results which have been generally

poor, although some reasonable control has occasionally been obtained (Conway and Forest, 1959; Hodgson, 1960; Martin, 1968). It may be that the inconsistencies could be due to variable detoxification via a free radical mechanism, indeed scorching of fronds by 4-CPA has been observed which was associated with poor control (Conway and Forrest, 1959). Synergism between 2,4-D and SCN has been reported in tomatoes (Hitchcock and Zimmerman, 1948). Thus, it may be that formulation of SCN, at a pre-determined mole to mole ratio depending on the free radical generating capacity of the target plant, may have the potential to improve the performance of the phenoxyacetic acid herbicides. Further work is necessary here in view of the widespread use of these herbicides. Under the conditions of the bracken pinnule bioassay scorching, aminotriazole produced different degrees of scorching at different sites. The plant and environmental factors governing the herbicide scorching responses are unclear. Asulam would not produce scorching unless at high concentrations. 2,4-D produces no scorching under the conditions of the assay. Thus, alternative whole or part-plant rapid assessment techniques would have to be developed to optimise the SCN ratios with such chemicals.

Using the pinnule bioassay for aminotriazole, a study made for possible additives other than SCN showed that thioacetamide was promising, however, it did not show up well in a field trial, due to perhaps low uptake. Because of its lower phytotoxicity than NH_4SCN , further work would be justified to fully evaluate the potential of thioacetamide as an additive in aminotriazole formulations.

By far the most successful additive for aminotriazole formulations found in the work of chapter 4 was the SCN ion, usually as the NH_4 salt. It was considered the most promising for improving aminotriazole formulations for bracken and other weed control. The phytotoxic response showed by I in the pinnule bioassay, although not surprising, was disappointing in that considerable in-vitro free radical scavenging ability had been previously demonstrated (Cook and Duncan, 1979). Since SCN was also toxic to bracken fronds, to a degree which varied at different sites, and to the underlying grass sward also, it was decided to look further at the behaviour of SCN and I when applied in their own right to plants, with an overall aim of maximising their role as free radical scavengers and minimising their own phytotoxic properties. As the work developed some interesting interactions between I and SCN and between the individual ions and plant constituents were revealed which merited some investigation. These aspects were covered in chapters 5 and 6.

The investigations of chapter 5 were designed to assess the role that endogenous SCN concentrations played on the selective herbicidal activity of I. The results showed that SCN is capable of inhibiting the oxidation of I by peroxidase systems and may affect the plants ability to form toxic I_2 . The levels of potential SCN found in resistant species are higher than in sensitive species, thus, this could contribute to the selectivity demonstrated by I in plants. The mechanism by which SCN acts would appear to possibly involve some non-competitive enzyme inhibition and free radical scavenging by SCN.

A major effect though is almost certainly the ability of SCN to reduce the newly formed I_2 back to I, either directly or through an influence on the overall oxidation-reduction balance of cell systems. Evidence was gathered to suggest other compounds present in plants, or some pesticidal additions, which could potentially affect both I_2 formation and/or persistence. Particularly relevant groups in terms of whole plants may be ascorbates and thiols and thioketones, in the form of glucosinolates, dithiocarbamates, glutathione and cysteine, etc. The relative importance of these groups would be difficult to ascertain, although the acknowledged high levels of glucosinolates (Van Etten *et al.*, 1969) and potential SCN levels (this study) of resistant species like cabbage and kale would seem to highlight their particular importance. It has to be considered that cell compartmentation may prevent these chemicals from exerting their activity against I_2 and their individual transport and metabolism may dictate their influence on I selectivity. As stated previously, areas of cell collapse have been demonstrated in both resistant and susceptible species in response to I application (Mynett and Wain, 1971). These authors also acknowledge the presence in crude leaf homogenates of components which will react with I_2 liberated on oxidation. The observed fall in-vivo of endogenous ascorbate levels on I addition (Hageman *et al.*, 1942; Mynett and Wain, 1973) supports the notion that these type of reactions are occurring. Promoters of peroxidase activity and oxidising agents may also contribute to the overall availability of I_2 . The contribution to selectivity of I accumulation in the leaves (Mynett and Wain, 1971) is without question. Differential I-peroxidase activities (Mynett and Wain, 1971), although

unlikely (Morrison and Bayse, 1973), cannot be wholly discounted due to the wide range of plant sources of peroxidase (Saunders et al., 1964) and the multiple forms of the enzyme found in a wide range of plant species (Jermyn, 1952; Macnicol, 1966) which may behave differently towards I oxidation. Time did not permit further study here. As discussed in chapter 6, I oxidation can take place by chemical means via the plant's photosynthetic apparatus. This will make the contribution from the plant's peroxidase activity less relevant in determining selectivity, however, the high potential SCN contents of plants like cabbage and kale could still be important here, due to their non-enzymic reduction of I_2 as illustrated in 5.c.

The simultaneous or pre-addition of some additives to I susceptible dwarf bean resulted in some degree of success in conferring, at least temporary, resistance to I. These studies tended to support the conclusions that several of the additives tested did play a role in determining the degree of I toxicity, hence contributed to selectivity. The fungicide zineb gave by far the most outstanding results in terms of preventing I toxicity. This, and the combination of other dithiocarbamate pesticides with I, is worthy of further investigation. A further pesticide combination which merits further study includes the effect of SCN on the activity of the nitrile herbicide ioxynil, bearing in mind that I may be liberated on ioxynil metabolism (Wain, 1964; Zaki et al., 1967). Zineb-ioxynil, SCN-bromoxynil and zineb-bromoxynil combinations would also be interesting.

The work of chapter 6 indicated the reasons for the variation within the halides and the pseudohalide SCN in their effects on plant growth. At high levels of application, I and SCN are toxic to beans

because of their ability to be oxidised to the toxic halogen or pseudohalogens. Through non-specific interactions with plant components, these products result in rapid dessication of the plants. Br, Cl and F are not oxidised and are non-toxic. At lower levels of application, I and SCN result in defoliation of dwarf bean plants. This was^{Probably} due to the effects of their respective oxidation products on endogenous IAA. The studies here showed that SCN could not be oxidised to the toxic moiety by the peroxidase system like I, thus, chemical oxidation to $(\text{SCN})_2$, probably by the plants photosynthesis apparatus, was required. Recent unpublished studies in this laboratory by Mr. D. Al-Jaff (1982) supports this. He has shown that SCN is not toxic to bean when the plants are kept in darkness or under low light intensity, whereas, I is toxic under both dark and light conditions.

The behaviour of SCN in plants is particularly interesting in that its oxidation to the toxic moiety does not take place so readily as with I and the interaction of $(\text{SCN})_2$ with IAA may possess something of a selective nature. These factors could make SCN addition an attractive mechanism for controlling IAA levels in plants. Of particular interest may be the effect that the addition of SCN has on the balance of IAA with ascorbic acid and sulphydryl compounds in plants. On one hand, SCN is oxidised to $(\text{SCN})_2$ which acts as a halogen, reacting with sulphydryl groups, IAA and ascorbic acid, etc. On the other hand, SCN, in the form of thiocyanic acid (HSCN) is itself a sulphydryl compound, belonging to a group which has often been related with IAA and also may have other roles in plant growth processes.

Also of interest for further investigation is the interaction between 2,4-D and SCN in plants, bearing in mind the activity of 2,4-D

as an auxin-type herbicide, the effects of SCN on IAA levels in plants and the possible sparing action of SCN on 2,4-D detoxification mentioned above.

APPENDIX 1

PRELIMINARY INVESTIGATION INTO THE MODE OF ACTION OF ASULAM

This appendix contains the experimental details and results of the preliminary studies into the mechanism of action of asulam as described in 2.A.

All principal reagents were as described elsewhere in this thesis.

A.1.1 Comparison of the influence of PABA, asulam and sulphanilamide on IAA oxidation by HRPO

With the exception of IAA, all reagents were prepared in 0.04M phosphate buffer at pH 6.0. IAA was dissolved in deionised water. Six ml of 5.94×10^{-4} M IAA and 2.5 ml of buffer or additive (7.8×10^{-3} M) were made up to 14.5 ml with buffer. The mixtures were allowed to equilibrate in a water bath at 35°C for 10 min prior to the addition of 0.5 ml of 0.5 mg/ml HRPO to start the reaction. After 5, 10 and 15 min, 2 ml samples were taken from each mixture and added to 2 ml of 0.05M NaAsO_2 to stop any further reaction. IAA was determined by the method of Tang and Bonner (1947). Each treatment was carried out in triplicate.

The percentage oxidation of IAA by HRPO in the presence and absence of PABA, sulphanilamide and asulam is shown in table A.1.1. All three additives increased the rate of oxidation. PABA was the most effective compound. Asulam enhanced the reaction more than sulphanilamide after 5 min but by 15 min this order was reversed.

Table 1.1.1 Influence of PABA, asulam and sulphanilamide on IAA
oxidation by HRPO

Treatment	% oxidation		
	time of reaction (min)		
	5	10	15
IAA	38 (1)	52 (1)	59 (1)
IAA + PABA	79 (1)	88 (1)	87 (1)
IAA + asulam	46 (2)	60 (1)	70 (2)
IAA + sulphanilamide	40 (1)	61 (2)	78 (1)

Values are expressed as percentage oxidation of IAA compared to a control which had no HRPO added. Figures in parentheses are standard deviations (n-1 D.F.).

A.1.2 Influence of equimolar concentrations of asulam, sulphanilamide and PABA on wheat growth

Wheat was grown as per 2.2.2.1 in the presence of equimolar concentrations of asulam (125-2500 $\mu\text{g}/\text{beaker}$), PABA (75-1500 $\mu\text{g}/\text{beaker}$) and sulphanilamide (95-1875 $\mu\text{g}/\text{beaker}$). Assessment was made after 6 days and analysis of the data carried out as per 2.2.2.1.

Asulam reduced wheat root lengths at all the levels tested, whereas PABA had no effect. In the case of sulphanilamide, the two highest concentrations (375 and 1875 $\mu\text{g}/\text{beaker}$) reduced root growth.

An increase in root length at the lowest sulphanilamide level (95 µg/beaker) was found to be non-significant. Shoot lengths followed the same general pattern as for roots; no stimulation was observed (table A.1.2).

Table A.1.2 Influence of equimolar concentrations of asulam, PABA and sulphanilamide on wheat growth

Treatment	Mean shoot length	Mean root length
control	100 (12)	100 (15)
asulam (125)	96 (6)	*** 52 (2)
asulam (250)	94 (8)	*** 46 (7)
asulam (500)	*** 57 (5)	*** 31 (7)
asulam (2500)	*** 13 (2)	*** 14 (12)
PABA (75)	107 (4)	98 (7)
PABA (150)	104 (3)	103 (10)
PABA (300)	104 (7)	106 (8)
PABA (1500)	102 (3)	89 (16)
sulphanilamide (95)	107 (7)	118 (16)
sulphanilamide (185)	105 (6)	96 (7)
sulphanilamide (375)	107 (5)	* 81 (10)
sulphanilamide (1875)	*** 78 (5)	*** 40 (5)

Assessment was made after 6 days. Shoot and root lengths are expressed as percentages of the control lengths. Figures in parentheses (length data) are standard deviations (n-1 D.F.). Treatment concentrations are in µg/beaker. Treatments significantly different from the control are indicated: * at the 5% level; ** at the 1% level; *** at the 0.1% level.

A.1.3 Influence of PABA on wheat growth when combined with a selected level of asulam or sulphanilamide

Levels of asulam (50 µg/beaker) and sulphanilamide (95 µg/beaker) calculated to be non-toxic to wheat were assayed in the presence and absence of PABA (1500 µg/beaker). The wheat was grown and assessed as above (A.1.2).

For asulam alone, the level employed was toxic to root growth. In the presence of PABA, this effect was completely overcome. No stimulatory effects from sulphanilamide, alone or in combination with PABA, was noted (table A.1.3).

Table A.1.3 Influence of PABA on wheat growth in combination with selected levels of asulam and sulphanilamide

Treatment	Mean shoot length	Mean root length
control	a 100 (6)	a 100 (5)
PABA(1500)	a 104 (4)	a 91 (5)
asulam(50)	a 97 (9)	b 64 (7)
sulphanilamide(95)	a 98 (9)	a 96 (8)
asulam(50)+PABA(1500)	a 102 (5)	a 98 (6)
sulphanilamide(95)+PABA(1500)	a 108 (6)	a 99 (4)

Treatment concentrations are in µg/beaker. Assessment was made after 6 days. Shoot and root lengths are expressed as percentages of the control lengths. Figures in parenthesis (length data) are standard deviations (n-1 D.F.).

Values with a similar letter, within each column, are not significantly different at the 5% level in Duncans Multiple Range test.

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